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1 **Thermal dynamics of ovarian maturation in Atlantic cod**

2 (*Gadus morhua*)

3

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21 **Abstract:** The timing and success of spawning in marine fish is of fundamental
22 importance to population persistence, distribution and, for commercial species,
23 sustainability. Their physiological processes of reproduction are regulated, in part, by
24 water temperature, and therefore changes in marine climate may have dramatic effects
25 upon spawning performance. Using Atlantic cod (*Gadus morhua*) as a case study, we
26 examined the links between water temperature, vitellogenesis and spawning time by
27 conducting extensive laboratory and field studies. Our experiments documented that
28 vitellogenesis generally starts at autumnal equinox, and that oocyte growth and
29 investment is greater in cod held at warmer temperatures. Furthermore, spawning
30 occurred earlier when oocyte growth was more rapid. The experimental results were
31 confirmed by measurements of oocyte growth collected from wild caught cod in
32 northern (Barents Sea) and southern (Irish and North Seas) populations. A model of
33 oocyte maturation was successfully developed to explain the results. This model was
34 consistent with published egg production curves of cod from the Barents Sea, North
35 and Irish Seas, considering *in situ* temperatures recorded by individual data-storage
36 tags on cod in those areas. These findings have considerable relevance for
37 future studies of fish recruitment in relation to climate change.

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40 **Keywords:** cod, vitellogenesis, temperature, light, spawning

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46 **Introduction**

47 Current scenarios of climate change are based on extensive analyses of a suite of
48 environmental variables and have led to the conclusion that many natural systems are
49 being affected by regional climate changes, particularly temperature increases (IPCC
50 2007). The focus on temperature has resulted in many studies examining the link
51 between species distributions and climate change (Parmesan and Yohe 2003). The
52 marine environment is no exception, with some authors suggesting that the southern
53 limit of species distributions are rapidly moving northwards (Perry et al. 2005).

54 While species distributions are likely to change, many populations will persist
55 under sub-optimal conditions and therefore it is also necessary to understand how
56 changes in marine climate will affect vital processes (Drinkwater 2005; Pörtner et al.
57 2008). This can be achieved by undertaking process-oriented and mechanistic studies,
58 and placing the results in the framework of metabolic ecology (Brown et al. 2004;
59 Sousa et al. 2008). For example, Pörtner et al. (2008) concluded that, based on studies
60 on eelpout (*Zoarces viviparous*) and Atlantic cod (*Gadus morhua*), temperatures
61 beyond pejus ('turning worse') are first felt at the whole organism level (due to
62 oxygen-limited thermal tolerance) followed by reductions in growth and reproductive
63 investment. Thus the physiological effects of extreme warming seas are likely to have
64 far-ranging effects on population dynamics (Brander 2007).

65 Atlantic cod is a species of high commercial and socioeconomic value that occurs
66 throughout the north Atlantic in waters predicted to be amongst those which show the
67 largest temperature rises in the world (Drinkwater 2005; IPCC 2007). Cod grow
68 faster, become fatter, mature at an earlier age, and are distributed further north with
69 increasing environmental temperature (Dutil and Brander 2003; Drinkwater 2005;
70 Sundby and Nakken 2008). The reproductive performance of cod is also sensitive to

71 temperature. Overall fecundity seems to increase with temperature (Kjesbu et al.
72 1998; Pörtner et al. 2001), but the quality of the eggs may be reduced beyond $\sim 10^{\circ}\text{C}$
73 (Pepin et al. 1997; Geffen et al. 2006; van der Meeren and Ivannikov 2006). The
74 physiological links between water temperature and reproductive success are likely
75 traceable to the aerobic demands of their large reproductive organs (up to 20% of
76 body weight) (Pörtner and Farrell 2008; Pörtner et al. 2008). The ovary of a spawning
77 cod is highly active sequestering vitellogenin from the plasma, i.e., during spawning
78 there is a significant increase in the transport of protein from the white muscle via the
79 liver to the high number of developing oocytes (Kjesbu et al. 1991, 1996).

80 In addition to the number and quality of eggs, the timing of spawning behaviour is
81 also critical to reproductive success (Wright and Trippel 2009). Cod spawn earlier in
82 the year in the warmer (more southerly) areas of the species' distribution (Brander
83 2005), which appears to be the result of the interaction between three main factors.
84 First, the environmental conditions suitable for larval development occur earlier in the
85 year in warmer seas (Planque and Fredou 1999). Second, the onset of sexual
86 maturation in cod is related to day length (Bromage et al. 2001; Norberg et al. 2004;
87 Davie et al. 2007). Finally, the temperature that cod experience during the vitellogenic
88 period influences the timing of egg release (Kjesbu 1994), with egg release being
89 delayed in colder waters.

90 Developing a clearer and more precise understanding of the exogenous factors
91 regulating reproductive development, and their interaction, is a necessary prerequisite
92 for predicting the effect of warming seas on reproductive success in marine fish. To
93 do so requires a comprehensive, process-oriented analysis based on existing
94 knowledge combined with new information from experimental and field studies. We
95 took advantage of recent advances in image analysis and ovary sampling techniques

96 (Kjesbu et al. 1996; Thorsen and Kjesbu 2001; Witthames et al. 2009) to track oocyte
97 growth in cod under experimentally controlled conditions. Our target was to
98 determine the underlying principles regulating the natural maturity cycle of cod under
99 different environmental conditions, and to establish an accurate and precise oocyte
100 growth curve, ideally applicable to all stocks. Gonad growth apparently commences
101 around the time of autumnal equinox (Woodhead and Woodhead 1965; Kjesbu 1991;
102 Davie et al. 2007) but more exact information is required to pin-point the time of
103 vitellogenesis initiation in the year. Likewise, the rate of development of oocytes
104 under different temperature regimes has not yet been adequately shown but modelled
105 using physiological principles (Kjesbu 1994). Secondly, we integrated this
106 understanding with temperature data collected using electronic archival tags on wild
107 cod under natural conditions to enable us to answer the question of how trade-offs
108 between body growth and reproductive performance are influenced in different
109 thermal environments, addressing in particular the effect of temperature on variation
110 in reproductive traits like fecundity and size-specific spawning time. Finally, the
111 overall spawning time model should as far as possible be simple to run, i.e., be based
112 on mechanistic principles rather than new raw data, and properly tested by consulting
113 published egg production curves for cod in different waters.

114

115 **Materials and methods**

116 **Laboratory study**

117 *Main protocol*

118 The experiment took place at the Institute of Marine Research (IMR) in Bergen
119 between 1 June 2005 and 26 January 2006 using reared local Norwegian Coastal cod
120 brought to the laboratory for acclimation in December 2004. The fish were produced

121 semi-extensively at the IMR marine pond facility Parisvatnet, Øygarden, west of
122 Bergen (Blom et al. 1994) in the spring of 2003, subsequently vaccinated against
123 vibriosis, and transported to IMR Austevoll Research Station in March 2004 for on-
124 growth. At arrival in Bergen an equal number of fish were placed into two identical
125 semi-rectangular 30-m³ outdoor concrete tanks (water depth: 1.8 m). The top of the
126 tank was covered by a net to reduce light intensity (by about 70%). Sea water was
127 continuously supplied from the bottom of the nearby deep fjord, after sand-filtration
128 and degassing, to refresh the water in each tank. In January 2005, all individuals were
129 anaesthetized (60 ppm benzocaine in oxygenated sea water) and sex determined by
130 gonad catheterization ('biopsied') using a Pipelle de Cornier® endometrial suction
131 curette (Witthames et al. 2009), and PIT-tagged. As expected (Svåsand et al. 1996),
132 all these 2-yr-old individuals were sexually mature; both running males and
133 maturing/running females were noted. As for the period at Austevoll, the fish were
134 given dry feed ([www. skretting.no](http://www.skretting.no): Amber Neptun) consisting of 52% protein, 18%
135 fat and 9.6% nitrogen-free extracts with a total energy content of 21.0 MJ•kg⁻¹. In late
136 spring 2005 a special broodstock feed (Vitalis Repro Cod) from the same producer
137 was used to speed up recovery after the completion of the first spawning season.

138 At the start of the experiment all fish were mixed together and randomly
139 reassigned to the same tanks used previously. In one of the tanks (n = 80) the water
140 was consistently maintained at ambient temperature (AT), i.e., at approximately 9-10
141 °C throughout the year, while in the other tank (n = 75) the temperature was gradually
142 reduced overnight to 5 °C, designated as low temperature (LT). Thus, the guidelines
143 of Schmidt-Nielsen (1983) for further Q₁₀ studies (see below) were followed strictly,
144 including that the experimental temperatures should be 'sufficiently far apart' but still
145 fall within the natural temperature range of the fish (Sundby 2000; Brander 2005).

146 Fish were handfed *ad libitum* three times per week (Kjesbu et al. 1991) using the
147 standard dry feed described above but no food was given 2-5 days before weighing of
148 the fish. Care was taken to stop feeding when appetite dropped markedly. The amount
149 of uneaten pellets (waste feed) was judged from the number of pellets remaining on
150 the tank bottom 1 h after feeding whilst the maximum stocking density was $8 \text{ kg}\cdot\text{m}^{-3}$.
151 The tank was vacuum-cleaned to remove any waste feed and excreta once a week.

152 Every month, 1 June (Day 0), 5 July (Day 35), 3 August (Day 64), 6 September
153 (Day 98), 6 October (Day 128), 2 November (Day 155), 6 December (Day 189) and
154 10 January (Day 224) all specimens were anaesthetized, weighed (W, 1 g) and
155 measured for total length (TL, 0.5 cm below). In total 75 LT and 74 AT individuals
156 (both genders) could be successfully followed over the whole experiment. Biopsies
157 were taken from Day 128 onwards to establish oocyte growth curves (Kjesbu 1994).
158 Initiation of spermiation was tested by hand stripping. Oocyte sizes, measured on Day
159 224, together with established oocyte growth rates were used to define the time for
160 termination of the experiment, with the aim that the groups would consist of both
161 prespawning ('prespawners') and spawning females ('spawners'). Consequently, all
162 specimens were exposed to a lethal dose of anaesthetic and killed on Day 240 (26
163 January) by a blow to the head. Total length and whole body weight was measured
164 and the whole gonad, liver and remaining viscera were carefully removed and
165 weighed (0.1 g). Apart from a few exceptions, only data from the females are
166 presented here.

167 ***Analysis of ovarian biopsies***

168 About 0.25 g of ovarian tissue (n = 344) was collected from each female on Day
169 128, 155, 189, 224 and 240, removing a total of ≤ 1 % referring to the final ovary
170 weight measured on Day 240. The samples were fixed in 3.6 % phosphate buffered

171 formaldehyde (Bancroft and Stevens 1996), stored and analysed automatically for
172 oocyte diameter $> 200 \mu\text{m}$ (Thorsen and Kjesbu 2001). Out of the 200 normal oocytes
173 (follicles) measured per sample the largest 10% was defined as the leading cohort
174 (LC) and the corresponding mean diameter (LC diameter) used to specify the maturity
175 phase (West 1990; Kjesbu 1994; Thorsen and Kjesbu 2001). Similar data on the
176 smallest 10% of oocytes were taken to reflect the smallest cohort (SC diameter), to
177 study hiatus (gap) development between previtellogenic ($\leq 250 \mu\text{m}$) and vitellogenic
178 oocytes ($> 250 \mu\text{m}$) (Sivertsen 1935) and thereby termination of oocyte recruitment.
179 Some caution should be expressed for data between 200-250 μm due to contrast
180 problems during the automated procedure. The vitellogenic oocyte distribution of cod
181 should be considered as unimodal and homogeneous throughout the ovary (Witthames
182 et al. 2009). The width of this distribution, reported as standard deviation ($SD_{\text{diam.}}$),
183 was included to strengthen the understanding of oocyte growth dynamics and to
184 indicate portion of eggs spawned, seen by a gradual fall in $SD_{\text{diam.}}$ (Kjesbu et al. 1990).
185 Hydrated or ovulated oocytes, used as spawning markers, were noted but not
186 measured. Individual fecundity was given from oocyte packing density (number of
187 oocytes $\cdot \text{g}^{-1}$), estimated from the mean diameter of all 200 oocytes, multiplied with
188 whole ovary weight, i.e., the Auto-diametric method (Thorsen and Kjesbu 2001). To
189 test for normal oocyte development a limited numbers of samples were processed
190 histologically using conventional protocols, i.e., Technovit® as embedding medium
191 and 2 % toluidine blue and 1 % sodium tetraborate as stain.

192 *Experimental definitions and calculations*

193 Data on body growth were split by gender and tank (LT and AT), while spawning
194 status (prespawners or spawners) was added as a third category when considering
195 reproductive information. Common expressions were applied in all calculations.

196 Growth analyses included: 1) specific growth rate (G, in percentage), $G = 100 \times (\ln W_2$
 197 $- \ln W_1) / (t_2 - t_1)$, where W_1 is initial weight at time t_1 and W_2 final weight at time t_2 ,
 198 and 2) daily length increment (DLI, in $\text{mm} \cdot \text{day}^{-1}$), $\text{DLI} = 10 \times (\text{TL}_2 - \text{TL}_1) / (t_2 - t_1)$
 199 (Svåsand et al. 1996), with TL_1 corresponding to total length at t_1 and TL_2 to total
 200 length at t_2 . To be able to compare overall G with previous studies, males were also
 201 included in the calculation.

202 Reproductive investment was defined as: 1) fecundity (F, number of vitellogenic
 203 oocytes); 2) relative fecundity (RF, number of vitellogenic oocytes \cdot whole body
 204 weight⁻¹); or 3) gonadosomatic index (GSI, $100 \times \text{gonad weight} \cdot \text{whole body weight}^{-1}$).
 205 Fish condition was presented either as liver (hepatosomatic) index (HSI, $100 \times \text{liver}$
 206 $\text{weight} \cdot \text{whole body weight}^{-1}$), or Fulton's condition factor (K, $100 \times \text{whole body}$
 207 $\text{weight} \cdot \text{total length}^{-3}$). Occasionally ovary-free weight (somatic weight) replaced
 208 whole body weight, marked with subscript s . The influence of experimental
 209 temperature on daily growth in LC diameter was established by: 1) common linear
 210 regression analysis at either the group (tank) or the individual level and 2) estimation
 211 of the Q_{10} value, $Q_{10} = (R_2/R_1)^{10/(T_2-T_1)}$ (Schmidt-Nielsen 1983) where estimated slopes
 212 (rates) from the previous regressions were labelled as $R_{1,LT}$ and $R_{2,AT}$ and
 213 corresponding temperatures as $T_{1,LT}$ and $T_{2,AT}$. Predictions of a new rate (R_{new}) at
 214 another temperature (T_{new}) was found after rearrangement of the standard Q_{10} formula:
 215 $R_{\text{new}} = R_{1,LT} \times Q_{10}^{(T_{\text{new}} - T_{1,LT})/10}$ (or $R_{\text{new}} = R_{2,AT} \times Q_{10}^{(T_{\text{new}} - T_{2,AT})/10}$) (Schmidt-Nielsen
 216 1983). Final maturation for the presently fixed oocytes was set to start (eccentric
 217 germinal vesicle (GV)) and end (GV breakdown) at a LC diameter of 875 and 1000
 218 μm , respectively, found after conversion (Thorsen and Kjesbu 2001) of fresh oocyte
 219 data (Kjesbu et al. 1996). The length of the vitellogenic period, i.e., from 250 to 875
 220 μm , was given as $625/R_{1,LT}$ and $625/R_{2,AT}$ (days).

221 Feeding ration (FR) was calculated for each of the eight successive periods
222 between fish measurements, and for the whole experiment as such. In the first
223 situation FR was calculated as the total amount of feed eaten during each period
224 divided by estimated total fish biomass in the tank midway in the period ($(\Sigma W_1 +$
225 $\Sigma W_2)/2$) and the number of days in question ($t_2 - t_1$). FR for the whole experiment
226 was weighted mean periodic feeding ration.

227 Experimental water temperature was reported as grand mean weekly temperature
228 based on 3-7, usually 5, measurements per week. Temperatures limited to the
229 vitellogenic period ($T_{vit.}$) were given separately.

230 **Field study**

231 *DST-recorded temperatures*

232 Information on temperatures experienced during the length of the maturation cycle,
233 recorded by data storage tags (DSTs), was acquired from previous projects studying
234 free-ranging cod. The temperature data were compiled from the longest DST records
235 available in the English Channel (Channel), southern North Sea and Irish Sea
236 (southern waters) and the Barents Sea (northern waters).

237 In the case of the northern individuals ($n = 6$), all appear in Godø and Michalsen
238 (2000), showing the following total length at release (tag number in parenthesis) in
239 March 1996: 64 (246), 74 (117), 72 (131), 65 (204), 73 (206) and 81 (44) cm.
240 Successful recording times were 12 ± 1 month. The last fish mentioned was tagged at
241 the spawning ground in Lofoten, i.e., considered to be sexually mature, while the
242 others were tagged at the Finnmark coast and mostly believed to be sexually
243 immature. However, the majority were likely to be sexually mature at recapture in
244 1997, seen by consulting the corresponding length-at-age key and maturity-at-age
245 ogive reflecting a probability of 73 – 93% (ICES Advisory Committee 2008). The

246 DST was attached externally and the temperature recorded (precision: ± 0.2 °C) in
247 weekly cycles of every 2 h for the first 6 days and every 12 h on the 7th day. In
248 southern waters DST data (accuracy: ± 0.1 °C; precision: 0.03 °C) were collected from
249 10 specimens tagged between 1999 and 2005 showing recording times comparable to
250 those given above for the Barents Sea cod but using a higher measuring frequency of
251 once per 10 min (Neat and Righton 2007). Data were collected from tags attached
252 either externally or implanted internally but the difference in site was considered
253 unimportant (Righton et al. 2006). Also, any between-year variation recorded was
254 considered negligible in relation to within-year fluctuation (Neat and Righton 2007).
255 Thus, to ease visual comparison, monthly-resolved data, including also for the cod in
256 the north, were plotted within a single year. The tagged cod in southern waters were
257 released in February, except for the largest fish measured, i.e., an 86-cm Channel cod,
258 being released in March. The total length for the other nine specimens ranged 47 – 66
259 cm. The examined Channel and North Sea cod showed 75 – 100 % probability of
260 being sexually mature (R.D.M Nash, IMR, Norway, Final Report, RASER (EU-
261 project Q5RS-2002-01825)) whilst the one examined from the Irish Sea certainly was
262 sexually mature (Armstrong et al. 2004).

263 *CTD-recorded temperatures*

264 IMR Barents Sea CTD (conductivity, temperature and depth) files were consulted
265 to indicate the effect of annual variation in environmental temperature on gonad
266 maturation. More specifically, the average temperature in August-September (1998-
267 2007) in the Vardø North Transect (72°15'N – 74°15'N, 31°E, depth: 50-200 m) was
268 compared with development in maturity stage as observed 6-7 months later.
269 Considered temperatures correlated well with bottom temperatures (R. Ingvaldsen,

270 IMR (personal communication, 2008)) in the centre of the cod distribution (Sundby
271 2000) just before or at initiation of vitellogenesis (Kjesbu 1991).

272 Vardø North Transect temperature was in one case contrasted with similar type of
273 information from DSTs. Transect temperature measured in August/September 1996
274 and January 1997 were averaged and related to average DST temperatures between 1
275 September 1996 and 1 February 1997 for each of the above-mentioned six Barents
276 Sea cod.

277 *Fish sampling and analyses*

278 Adult cod were collected both from northern and southern waters using very much
279 the same protocols, although in the last situation the sampling was spread over several
280 days, including into the spawning season. Northern fish were worked up just after
281 landing of the catch while this procedure took place onboard for the southern fish.

282 The sampling in the northern area was part of the regular 'Andenes fecundity time
283 series' (Kjesbu et al. 1998; Thorsen et al. 2006), i.e., examining females (n = 486)
284 captured by commercial vessels over a period of 1-2 days in early-mid March
285 (calendar day 57-74) off the Vesterålen region, Northern Norway (69°19'N 16°09'E).
286 The Andenes study was limited to Barents Sea (Northeast Arctic) cod, excluding 5-
287 30% of the material classified, from the otolith, as Coastal cod. Otoliths were also
288 used for reading of age and spawning zones (Rollefsen 1934). Presently eight years
289 spanning from 1999 to 2008 were included. Thus, only ovarian samples analysed after
290 the introduction of the Auto-diametric method (see above) were considered.

291 Generally, close to 100 % of the fish were prespawners, i.e., only a few spawners
292 were detected based on the presence of hyaline or ovulated oocytes. In 2006 an extra
293 sample was taken in mid-February (calendar day 43-44: 'early 2006') to be compared
294 with the standard sample (calendar day 66-67: 'late 2006'). To further evaluate

295 representativeness of the adopted sampling scheme, year-specific length-at-age data
296 were contrasted with similar type of data available from the statutory Lofoten-
297 Vesterålen survey in mid-March - late April (Korsbrekke et al. 2001; ICES Advisory
298 Committee 2008). However, as these data were not resolved by sex, the present
299 comparison was limited to 2005-2007, i.e., in years when the Andenes program was
300 extended to include males. Each fish was physically characterized by its total length
301 (1 cm below), weight of the whole body (10 g), ovary, liver and viscera (1 g). Viscera
302 comprised of all organs left in the body cavity after removing the ovary and liver, and
303 as much as possible of the oesophagus. Any stomach content was judged by dominant
304 species. Cases where the stomachs were devoid of contents were noted as a special
305 category.

306 Fish from the southern area were collected in 2004 in the central North Sea (n =
307 41) and the eastern Irish Sea (n = 38) from catches made with IBTS gear (North Sea)
308 or a commercial rock hopper trawl (Irish Sea). Due to low catchability, the collection
309 of specimens was stretched over 34 days (22 January – 24 February) and 10 days (10
310 – 19 February), respectively. Hence special emphasis was placed on the establishment
311 of relevant maturity standardisation techniques, as detailed in the Result Section. Only
312 total lengths (1 cm below) along with measured oocyte data (see below) were
313 considered. Spawners showed either hydrated/ovulated oocytes, or recent post-
314 ovulatory follicles. The latter structures were detected in resin sections specially
315 aimed for this purpose, i.e., using PAS – Mallory's trichrome stain (Witthames et al.
316 2009). As previously, final oocyte maturation was set to be introduced at 875 μm , as
317 there were no indications of any deviation.

318 *Analysis of ovarian sub-samples*

319 All sampling was carried out from the right ovarian lobe using either a plastic
 320 pipette with a wide opening (IMR) or a standardized Wiretroll II pipette (Bohit)
 321 (Cefas) (Witthames et al. 2009). The corresponding LC diameter was added to the
 322 established fish database and supplemented with mean oocyte diameter for Barents
 323 Sea cod to estimate fecundity. Variation in LC diameter across the whole ovary was
 324 about $\pm 10 \mu\text{m}$ (SE) tested on seven Barents Sea cod (total length: 89-111 cm) in the
 325 standard Andenes program (2003), cf. also Fig. 1 in Witthames et al. (2009).
 326 Calibration tests performed between institutes showed that the two image analysis
 327 programs used were fully compatible (Witthames et al. 2009).

328 *Field-related definitions and calculations*

329 The following relationship was established between weight of viscera with empty
 330 stomach (VW_{empty} , g) and total length (TL, cm) for Barents Sea fish:

331

$$332 \quad (1) \quad VW_{\text{empty}} = 1.38 \times 10^{-5} \times TL^{3.722} \quad (r^2 = 0.940, \text{df} = 1,56, p < 0.001, \text{TL: } 58\text{-}124 \text{ cm}).$$

333

334 Prior to antilogarithm, the constant had a logarithmic value of -11.19 and an
 335 associated SE of 0.57. SE for the exponent was 0.126. No year effect was noted (late
 336 2006 vs. 2008) (slope: $p = 0.074$; intercept: $p = 0.178$) (ANCOVA). Thus, whole body
 337 weight (W , g) could be corrected ($W_{\text{corrected}}$, g) for varying stomach content:

338

$$339 \quad (2) \quad W_{\text{corrected}} = W - (VW - VW_{\text{empty}}),$$

340

341 where VW is recorded weight of viscera (g). At a given length, based on all standard
 342 Andenes samples, expected body weight (W_{expected} , g) was:

343

344 (3) $W_{\text{expected}} = 2.76 \times 10^{-3} \times \text{TL}^{3.266}$ ($r^2 = 0.970$, $\text{df} = 1,445$, $p < 0.001$, $\text{TL}: 54\text{-}128$ cm).

345

346 The constant showed a logarithmic value of -5.89 with SE equal to 0.12. The

347 exponent SE was 0.027.

348 Based on these approaches, viscera condition, C_{viscera} , and fish condition C_{weight} ,

349 were defined as: $C_{\text{viscera}} = \text{VW}/\text{VW}_{\text{empty}}$ and $C_{\text{weight}} = W_{\text{corrected}}/W_{\text{expected}}$. The latter

350 expression was used to handle problems with size-dependency in condition (Scott et

351 al. 2006), simultaneously cancelling out any noise in the data caused by varying

352 stomach content. Specific growth rate was found from the standard formula (see

353 above) defining W_1 and W_2 as $W_{\text{corrected, age 8}}$ and $W_{\text{corrected, age 9}}$, respectively, and $(t_2 - t_1)$

354 as 365 days, i.e., studying separate cohorts (Dutil and Brander 2003). Annual total

355 length increment (ALI) was estimated as total length divided by the corresponding

356 age ($\text{cm} \cdot \text{year}^{-1}$).

357 **Light cycle**

358 The duration of daylight from 1 June 2005 to 26 January 2006 (i.e. the present

359 experimental period) at Guernsey (49°27'N, 02°33'W) (Channel), Isle of Man

360 (54°15'N, 04°30'W) (Irish Sea), Bergen (60°24'N, 05°18'E) (Experiment) and Bear

361 Island (74°27'N, 19°02'E) (Barents Sea) was taken from the Astronomical

362 Applications Department of the U.S. Naval Observatory, USA

363 (<http://aa.usno.navy.mil>). For plotting purposes, the number of minutes was

364 transformed into decimal fraction of an hour. Total duration refers to when any

365 portion of the sun is above the horizon. This was found for all days corresponding to

366 experimental measurements days ($n = 9$) but adding summer and winter solstice and

367 autumnal equinox.

368 **Statistics**

369 All statistical analyses were performed with Systat® 12 and the graphs produced
370 with SigmaPlot® 10. Prior to any statistical test, each subset of data was examined for
371 normal distribution by the Shapiro-Wilk test and the Anderson-Darling test (default
372 options). For proportions normality was in some cases achieved by arcsine
373 transformation (Sokal and Rohlf 1981). Equality of variances was tested with the F-
374 test (incl. the Levene test). Coefficient of variation (CV) was presented as
375 $100 \times \text{SD} / \text{mean}$ (%). Tests between or among groups included both nonparametric
376 (Mann-Whitney test and Kruskal-Wallis test) and parametric methods (Student t-test).
377 For ANCOVA the assumption of homogeneity of slopes was tested prior to any test
378 on intercepts, using ln-transformed data when required. In regression analysis,
379 standard error was attached to each regression coefficient and r^2 replaced with
380 adjusted r^2 at low number of observations. For multiple regressions the entry of an
381 independent variable was based as far as possible on biological relevance consulting
382 experimental findings when establishing field models. Unless specially mentioned,
383 any predictor adopted showed a significant contribution (an absolute value of $t > 2.0$)
384 and 'tolerance' > 0.1 , the latter to exclude highly correlated predictors (Systat
385 Software 2007). The Akaike Information Criterion (AIC) was consulted when
386 appropriate, searching for the lowest AIC (Systat Software 2007). In 'tracking
387 studies' on the same experimental individuals across time (i.e. balanced design)
388 observed changes were tested with Linear Mixed Models (LMM) and/or Repeated
389 Measure ANOVA. To clarify the specific influence of a given category at specific
390 points within such time series, Hypothesis Test (Effects) ANOVA was used. For
391 LMM fixed factors were tank, time (month), gender (if relevant) and tank \times month, and
392 the random factor set to be fish \times tank. The default first-order autoregressive structure
393 was included to adjust for autocorrelation with time. Resulting adjusted p -values for

394 fixed effects were consulted and presented. Rejection of null hypothesis was always
 395 set at $p < 0.05$.

396

397 **Results**

398 **Laboratory study**

399 *Water temperature and food intake*

400 Grand mean (SD) temperature during the length of the experiment (240 days) was
 401 5.05 (0.49) at LT (low temperature) and 9.33 (0.59) °C at AT (ambient temperature).
 402 Variation in temperature within a week was typically ± 0.5 °C. The smallest between-
 403 tank difference in temperature was 2.9 °C, the largest 5.2 °C (Fig. 1a). Daily
 404 temperature showed evidence of synchrony between tanks ($r = 0.474$, $p < 0.001$).

Fig 1 near here

405 Weighted mean feeding ration (FR) for the eight measurement periods at LT and
 406 AT was 0.226 and 0.244 dry feed•g wet fish⁻¹•day⁻¹, respectively, i.e., not
 407 significantly different ($p = 0.774$) (Student t-test). Periodic FR declined over time in
 408 both tanks (Fig. 1b). There were indications that the AT fish took more feed initially
 409 (Day 0-64), but later on the appetite in the two tanks was similar. There was a
 410 transitory drop in interest in food between Day128 and 155, coinciding with initiation
 411 of vitellogenesis (see below).

412 *Fish growth and condition*

413 Individual growth in body weight showed evidence of tank ($p = 0.011$), month ($p <$
 414 0.001), gender ($p = 0.017$) and tank×month ($p < 0.001$) effects (LMM). Despite this
 415 overall specific growth rate (G) did not vary statistically between tanks but overall
 416 daily length increment (DLI) did vary showing the highest figures for AT (Table 1).
 417 Fish at LT and AT were similar in size at the start of the experiment, both in terms of

Table 1 near here

418 mean length and weight (Table 1). After 224 days their mean lengths were statistically
419 different (Table 1).

420 More in-depth analyses demonstrated that the specific growth rate varied
421 periodically. Both tanks demonstrated a strong positive relationship between mean
422 periodic G and periodic FR (LT: $r^2_{\text{adj.}} = 0.889$, $df = 1,6$, $p < 0.001$; AT: $r^2_{\text{adj.}} = 0.829$,
423 $df = 1,6$, $p = 0.001$, both genders). The fall in mean periodic G (Fig. 1c) essentially
424 mimicked the one for FR (Fig. 1 b) whilst the pattern of change across time for G
425 differed between tanks ($p = 0.007$) (Repeated Measures ANOVA). There was
426 evidence of a trade-off between initial body weight and subsequent growth rate (LT: r
427 $= -0.448$, $p = 0.009$; AT: $r = -0.382$, $p = 0.018$). Around the time of initiation of
428 spawning (see below) G generally became negative (Fig. 1c).

429 AT fish developed a significantly lower condition factor (K) than those at LT (Fig.
430 1d). The interaction month \times tank was highly significant ($p < 0.001$) (Repeated
431 Measure ANOVA). As a consequence, mean K at LT and AT became increasingly
432 significantly different (Day 0: $p = 0.057$; Day 35-98: $p \leq 0.017$; Day 128-240: $p <$
433 0.001) (Hypothesis Test ANOVA). Analysis of somatic condition factor (K_S) gave a
434 similar answer for the last measurement point (Day 240) (LT vs. AT prespawners: $p <$
435 0.001) (Student t-test).

436 ***Initiation of spawning***

437 A higher proportion of fish in AT (34%) compared to fish in LT (6%) started to
438 spawn on Day 240 indicated by either running eggs or hydrated oocytes. All ovaries
439 were in a normal state. Freely running ('spermiating') males were first noted on Day
440 224.

441 ***GSI, HSI, and fecundity regulation***

442 Prespawners held at AT showed, on average, a significantly higher gonadosomatic
 443 index (GSI), fecundity (F) and relative fecundity (RF) than their LT counterparts at
 444 Day 240 but a significantly lower hepatosomatic index (HSI) (Table 1). Only the LT
 445 regime showed evidence of any influence of maturity status, represented by LC
 446 diameter, on somatic relative fecundity (RF_S), but fish in both regimes developed a
 447 negative trend in RF_S with increasing LC diameter (LT: $r = -0.518$, $p = 0.003$; AT: $r =$
 448 -0.271 , $p = 0.189$) (Fig. 2). Exclusion of a statistical outlier at AT (Fig. 2) associated
 449 with an uncertain measurement did not influence the conclusion. AT prespawners
 450 demonstrated a significantly higher LC diameter-specific RF_S than LT prespawners
 451 (intercept: $p < 0.001$; slope: $p = 0.458$ (0.604, without outlier)) (ANCOVA).

Fig 2 near here

452 Testing the temporal influence of body size on fecundity, multiple regression
 453 analysis consistently revealed no significant effect of TL ($p \gg 0.05$) when W was
 454 used as the other independent variable. Hence, this type of analysis did not expose any
 455 condition effect as such on fecundity. Use of W as the only independent variable
 456 explained up to 36% of the variance (r^2) in F for LT and 40% for AT, referring to W
 457 on Day 224 and 240, respectively (Fig. 3). The level of significance was, however,
 458 rather similar throughout the experiment for AT ($0.001 < p < 0.004$), but steadily
 459 increasing for LT (p falling from 0.054 to 0.001). Inclusion of vitellogenic LC
 460 diameter (see below) contributed significantly to the regression for LT ($p \leq 0.046$) but
 461 not so for AT ($p \geq 0.077$) (Fig. 3). In the first case r^2 reached 0.681 on Day 240. The
 462 relative influence of LC diameter versus W on F, taken as the ratio of the
 463 corresponding absolute standard coefficients, increased in the case of LT from about
 464 50% on Day 155 to about 75% on Day 240 but was rather stable around 40% for AT.
 465 In all cases W contributed positively to F while LC diameter negatively. The

Fig 3 near here

466 respective F formulae (millions) based on W (g) and LC diameter (μm), all referring
 467 to Day 240, for LT and AT were:

468

469 (4) $F = 3.08(\text{SE} \pm 0.74) + 9.87 \times 10^{-4}(\text{SE} \pm 1.61 \cdot 10^{-4}) \times W - 4.14 \times 10^{-3}(\text{SE} \pm 0.92 \times 10^{-3}) \times$
 470 LC (LT, $r^2 = 0.681$, $df = 1,25$, $p < 0.001$)

471

472 (5) $F = 2.70(\text{SE} \pm 0.76) + 8.46 \times 10^{-4}(\text{SE} \pm 2.16 \times 10^{-4}) \times W - 2.12 \times 10^{-3}(\text{SE} \pm 1.28 \times 10^{-3}) \times$
 473 LC (AT, $r^2 = 0.481$, $df = 1,18$, $p = 0.004$).

474

475 For the sake of comparison LC was withheld in Eq. (5) despite its insignificant
 476 statistical contribution: at LC = 500 μm for a standard female of 3500 g the fecundity
 477 was 3% higher at AT than at LT but this difference increased to 23% at LC = 800 μm .

478 Data on oocyte diameter frequency distributions showed differences in oocyte
 479 recruitment dynamics both between tanks and between prespawners and spawners.
 480 The width of the oocyte distribution ($SD_{\text{diam.}}$) increased markedly over time but
 481 significantly more in AT prespawners than in LT prespawners (Day 189: $p = 0.163$;
 482 Day 224: $p = 0.017$; Day 240: $p < 0.001$) (Student t-test) (Fig. 4a). A comparable
 483 situation appeared between AT prespawners and spawners (Day 189: $p = 0.003$; Day
 484 224: $p = 0.013$), but the difference disappeared (Day 240: $p = 0.393$) when the
 485 spawners experienced a fall in $SD_{\text{diam.}}$ related to initiation of spawning (Student t-
 486 test). This analysis was not testable at LT. Indications of differences in $SD_{\text{diam.}}$
 487 between AT prespawners and spawners appeared early on (Day 155: $p = 0.051$) but
 488 not so between LT and AT prespawners (Day 155: $p = 0.820$) (Mann-Whitney test).
 489 When ignoring the subdivision into prespawners and spawners the pooled $SD_{\text{diam.}}$ data
 490 showed no differences between the two temperature regimes on Day 155 ($p = 0.330$)

Fig 4 near here

491 (Mann-Whitney test) but diverged by Day 189 ($p = 0.009$) and 224 ($p < 0.001$)
 492 (Student t-test). Corresponding data on the smallest cohort of oocytes (SC) revealed
 493 that the process of oocyte recruitment had ceased from Day 189, i.e., mean SC was
 494 then well above $250 \mu\text{m}$ (Fig. 4b), though a few individuals apparently recruited
 495 oocytes all the way up to Day 224. Any difference in SC development between LT
 496 and AT prespawners could not be fully confirmed (Day 155-240, $p > 0.062$) (Student
 497 t-test) but examples of such differences existed when contrasting AT prespawners and
 498 spawners (Day 155: $p = 0.504$; Day 189: $p = 0.009$; Day 224: $p = 0.054$; Day 240: $p =$
 499 0.001) (Mann-Whitney test). The pooled SC data demonstrated tank differences on
 500 Day 155 ($p = 0.009$) but not later on ($p > 0.687$) (Mann-Whitney test).

501 *LC oocyte diameter as maturation criterion*

502 The observation that AT females showed a reasonable mixture of prespawners and
 503 spawners on the last day of the experiment made it possible to test the assumption that
 504 individuals with larger LC diameter spawn first. This appeared to be generally true:
 505 on Day 224 females that subsequently spawned within the next two weeks showed on
 506 average a LC diameter of $694 (SE \pm 17) \mu\text{m}$ compared to $585 (SE \pm 16) \mu\text{m}$ for those
 507 that did not spawn within that period of time, i.e., a significant difference ($p < 0.001$,
 508 Student t-test).

509 *Overall oocyte growth rate*

510 LT and AT females tracked over time showed significantly different oocyte growth
 511 trajectories ($p < 0.001$, Repeated Measures ANOVA) resulting in significant
 512 differences in mean LC diameter (LC_{group}) at late vitellogenesis (Fig. 5). More
 513 explicitly, the two data sets differed statistically from Day 189 onwards (Day 128: $p =$
 514 0.521 , Day 155: $p = 0.075$, Day 189: $p = 0.011$, Day 224: $p = 0.005$ and Day 240: $p =$
 515 0.003) (Mann-Whitney test or Student t-test). Dropping spawners from the analysis,

Fig 5 near here

516 caused the observed differences on Day 189 and 224 to disappear ($p = 0.298$ and
 517 0.154 , respectively) and nearly so on Day 240 ($p = 0.048$). On Day 128 10% of the
 518 LT females and 6% of the AT females contained developing oocytes (LC diameter $>$
 519 $250 \mu\text{m}$). This figure increased sharply to 66% and 76% on Day 155, respectively.
 520 Hence, in relative terms more AT females entered vitellogenesis between Day 128
 521 and 155. The detailed individual tracking study revealed an example of extreme slow
 522 oocyte growth. Exclusion of this LT female did not affect the above statistical
 523 conclusions. LC_{group} diameter (μm) increased between Day 128 and 240 (Fig. 5) as:

524

525 (6) $\text{LC}_{\text{group}} = 3.43(\text{SE} \pm 0.20) \times \text{ED} - 197(\text{SE} \pm 39)$ (LT, $r^2 = 0.990$, $\text{df} = 1,3$, $p < 0.001$)

526

527 (7) $\text{LC}_{\text{group}} = 4.10(\text{SE} \pm 0.15) \times \text{ED} - 281(\text{SE} \pm 30)$ (AT, $r^2 = 0.996$, $\text{df} = 1,3$, $p < 0.001$),

528

529 where ED is elapsed days since Day 0. The use of a power function in place of a
 530 linear function increased r^2 even closer to 1, but had no practical implications. The
 531 typical oocyte growth rate (R) of AT females was 19.5% higher than for LT females,
 532 i.e., 3.43 at LT vs. $4.10 \mu\text{m} \cdot \text{day}^{-1}$ at AT.

533 *Individual oocyte growth rate*

534 Studies of individual LC diameter data showed that the time of entrance to
 535 vitellogenesis in the autumn influenced when each female would likely start to spawn
 536 in the subsequent spring. This was most clearly seen for the earliest and latest
 537 spawners: females with the largest LC diameter on Day 240 were all vitellogenic on
 538 Day 155 (LC diameter $> 380 \mu\text{m}$) while none of the females with the smallest LC
 539 values on Day 240 had yet commenced vitellogenesis on Day 155 (LC diameter ≤ 250
 540 μm). A regression analysis on the complete individual-based data sets indicated that

541 predicted spawning time for both temperature regimes showed similar dependence on
542 the time of entrance to vitellogenesis (intercept) and subsequent oocyte growth rate
543 (slope) (R), i.e., LC diameter on Day 155 and R influenced final LC diameter (Day
544 224) by 57-58% and 42-43 %, respectively. A few out of the tracked females deviated
545 from the rest by showing a fall in R past Day 155. Likewise, some apparently had a
546 temporarily increased R, mainly between Day 224 and 240, cf. appearance of
547 spermiating males. Nevertheless, the high individual stability in R preceding the
548 process of egg release was confirmed by separate regression analysis (LT: mean $r^2 =$
549 0.979 (SE ± 0.010), AT: mean $r^2 = 0.993$ (SE ± 0.001)), where each fish was
550 represented with four successive LC diameter measurement points. In one single case
551 the r^2 was much lower, i.e., 0.717. Subsequent histology showed a fully normal ovary.

552 The average individual R per tank was 3.56 (SE ± 0.15) and 4.21 (SE ± 0.12)
553 $\mu\text{m}\cdot\text{day}^{-1}$, i.e., about 3-4 % higher compared to the corresponding group-based
554 parameter values given in Eqs. (6) and (7), respectively. The R appeared to be
555 statistically independent ($p = 0.928$) on when the females entered vitellogenesis in the
556 autumn, being testable for AT (middle vs. late entrance, Mann-Whitney test).

557 Estimated R values reflected a general vitellogenic Q_{10} value of 1.44 (1.47) and a
558 length of the vitellogenic period of 176 (182) days at LT and 148 (152) days at AT,
559 with the results from Eq. (6) and (7) given in parenthesis. Thus, the respective ovaries
560 matured typically for 6 and 5 months before initiation of spawning, or stated in
561 another way, LT females showed a delay in spawning time of one month compared to
562 AT females. Based on these findings R_{new} ($\mu\text{m}\cdot\text{day}^{-1}$), i.e., the oocyte growth rate at
563 another environmental temperature (T_{new}) than the present ones can be predicted by
564 the following expression:

565

566 (8) $R_{\text{new}} = 4.21 \times 1.44^{(T_{\text{new}} - 9.60)/10}$.

567

568 As noted, input data are from AT and the Q_{10} value refers to vitellogenic females. The
 569 alternative use of input data from LT, would, logically, give the same result.

570 ***Size-specific oocyte growth***

571 Only AT females showed a significant relationship between LC diameter on Day
 572 224 and body weight and length measurements during the course of the experiment.

573 Of the two explanatory variables, W had, with the exception of Day 64-98, a higher
 574 predictive power than TL but both r^2 showed generally falling values with time (TL:
 575 Day 0: 0.34, Day 224: 0.19; W: Day 0: 0.37, Day 224: 0.27). All these regressions
 576 were significant (TL: $p \leq 0.007$; W: $p \leq 0.001$) but none for LT (TL: $p \geq 0.653$; W: p
 577 ≥ 0.082). Exclusion of the above-mentioned LT female with extremely slow oocyte

578 growth had no statistical relevance. All slopes were positive, except for two LT

579 outputs, which were considered irrelevant due to their insignificant nature. In

580 consequence, those AT females that turned out to be spawners before the end of the

581 experiment had on Day 0 a significantly larger body size than their accompanying

582 prespawners (W: $p = 0.023$ (Table 1); TL: $p = 0.026$ (mean: 56.0 vs. 53.6 cm))

583 (Student t-test). These prespawners, however, compared to the other time periods

584 tested ($p \geq 0.677$), showed indications of a higher specific growth rate between Day

585 35 and 98 ($0.171 < p < 0.181$) (Mann-Whitney test). This probably contributed to the

586 finding that AT spawners and prespawners became just insignificantly different in

587 mean body weight on Day 224 (Table 1) ($p = 0.060$) while their corresponding mean

588 lengths (65.0 vs. 63.8 cm) evidently had turned statistically similar ($p = 0.365$)

589 (Student t-test). Too few LT spawners existed for such a test. More comprehensive

590 multivariate analyses did not locate any other additional variables, such as

591 hepatosomatic index or expressions of body growth (G and DLI), which significantly
 592 increased the understanding of variation in prespawning LC diameter.

593 The underlying reason for any potential influence of body size on prespawning LC
 594 diameter was tested by regressing oocyte growth rate (R , $\mu\text{m}\cdot\text{day}^{-1}$) on initial length
 595 with no relationship in the case of LT ($p \gg 0.05$) but noticeably so for AT (Fig. 6):

Fig 6 near here

596

597 (9) $R_{\text{size}} = 10.42 \times 10^{-2} (\text{SE} \pm 3.40 \times 10^{-2}) \times \text{TL} - 1.50 (\text{SE} \pm 1.87)$ (AT, $r^2 = 0.232$, $df =$
 598 $1,31$, $p = 0.005$, $46 < \text{TL} (\text{Day } 0) < 61 \text{ cm}$).

599

600 For clarity, R was relabelled as R_{size} . Using initial body weight instead of length as the
 601 predictor gave similar results. This significant relationship persisted until both Day
 602 128 and 224 when studying the same individuals:

603

604 (10) $R_{\text{size}} = 8.24 \times 10^{-2} (\text{SE} \pm 2.94 \times 10^{-2}) \times \text{TL} - 0.90 (\text{SE} \pm 1.83)$ (AT, $r^2 = 0.202$, $p =$
 605 0.009 , $51 < \text{TL} (\text{Day } 128) < 68 \text{ cm}$).

606

607 (11) $R_{\text{size}} = 8.02 \times 10^{-2} (\text{SE} \pm 2.85 \times 10^{-2}) \times \text{TL} - 0.97 (\text{SE} \pm 1.85)$ (AT, $r^2 = 0.204$, $p =$
 608 0.008 , $53 < \text{TL} (\text{Day } 224) < 70 \text{ cm}$).

609

610 These two latter regressions were included due to practical applications in the field
 611 study below. Next, the following formula was established including both the above
 612 temperature (Eq. (8)) and body size effect (Eq. (9), (10) or (11) depending on the time
 613 in the autumn):

614

615 (12) $\text{LC}_2 = R_{\text{size}} \times 1.44^{\left(\frac{T_{\text{new}} - 9.60}{10}\right)} \times (t_2 - t_1) + \text{LC}_1$.

616

617 Thus, measured developing LC diameter (LC_1) on any day t_1 can in effect be
618 transferred (standardised) to developing LC diameter (LC_2) on day t_2 in a warm
619 temperature situation resembling the one of the AT regime.

620 **Field study**

621 *DST-recorded temperatures in the different waters*

622 Temperature information gathered from DSTs showed that adult cod in the
623 northern and southern waters stayed in highly different temperatures throughout the
624 year but without any evident trend in selected temperature by fish size. Monthly
625 temperature profiles from the Channel, North Sea and Irish Sea clearly differed from
626 those from the Barents Sea (Fig. 7). After the supposed spawning season, the southern
627 category went into significantly warmer water while those belonging to the northern
628 category generally entered cooler water, including temperatures below zero. Both
629 categories showed less variation around expected time of spawning, but particularly
630 the northern one, i.e., between March to May the temperatures experienced by the
631 present individuals of Barents Sea cod were concentrated around 4 °C, which was far
632 below the introduced threshold value of 9.6 °C, thought to imply impaired spawning,
633 if met or exceeded (Fig. 7). This was not the case for the three southern stocks
634 showing examples of individuals quite close to this critical line in the months of
635 interest, i.e., January-March. The data indicated that the overall temperature between
636 1 September and 1 February, assumed to overlap to a large extent with the period of
637 vitellogenesis, was centred on 2 °C (range: 1 – 3.5 °C) and 11 °C (range: 9 – 13 °C) for
638 cod in the north and south, respectively (Fig. 8). There was no obvious fish size
639 dependency as analysed within the Channel, North Sea and Barents Sea cod stocks
640 (for the Irish Sea only one fish was recorded).

Fig 7 near here

Fig 8 near here

641 ***Barents Sea data base***

642 Data from this area included physical descriptors (total length, viscera condition
643 (C_{viscera}) and body condition (C_{weight})) (Table 2) and CTD-recorded temperature in
644 August-September (Vardø North Transect, T_{VN}) (Fig. 9). All three physical
645 descriptors demonstrated significant annual variations within the standard time series
646 ($p < 0.001$) (Kruskal-Wallis test), although the length data (54 – 128 cm) should be
647 treated with some caution due to likely examples of non-random sampling. The early
648 and late (standard) 2006 samples also deviated significantly in length ($p = 0.032$) and
649 viscera condition ($p < 0.001$) but not in body condition ($p = 0.172$) (Mann-Whitney
650 test). Viscera condition was included to reflect feeding activity finding two major
651 peaks, 2003 and late 2006 (Fig. 9).

Table 2 near here

Fig 9 near here

652 To assess the representativeness of the various data, our fish measurement data
653 were first compared with similar survey data but also with present experimental
654 growth data, followed by a study on CTD data in relation to similar DST data, finding
655 some deviations. The comparison with the extensive length-at-age database from the
656 Lofoten-Vesterålen survey showed that the presently sampled cod, focusing on the
657 main age groups 8 and 9 (combined sexes), were consistently 5-10 % larger between
658 2005 and 2007. Corresponding specific growth rate (females only) varied typically
659 from about $0.05 \text{ \%} \cdot \text{day}^{-1}$ within the period 1999-2005 to about $0.12 \text{ \%} \cdot \text{day}^{-1}$ within the
660 period 2006-2007. Thus, indicated growth in body weight was roughly half, or less, of
661 the above experimental values. Transect temperature during the autumn of 1996, 3.1
662 $^{\circ}\text{C}$, was about $1 \text{ }^{\circ}\text{C}$ higher than the corresponding average DST temperature. The
663 individual range in DST temperature (Fig. 8) showed, however an overlap with this
664 CTD record.

665 ***Fecundity regulation in the Barents Sea cod***

666 The pooled analysis on standard Andenes samples (i.e., excluding early 2006)
 667 showed that the fecundity represented by F (millions) was significantly influenced (p
 668 < 0.001) by TL (cm), C_{weight} (without unit), LC diameter (μm) and T_{VN} ($^{\circ}\text{C}$):

669

670 (13) $F = 1.95 \times 10^{-4} \times \text{TL}^{3.726} \times C_{\text{weight}}^{1.729} \times \text{LC}^{-1.141} \times T_{\text{VN}}^{0.325}$ ($r^2 = 0.921$, $df = 1, 445$, $p <$
 671 0.001).

672

673 The constant had a logarithm value of -8.54 with SE 0.65. The SE of the exponents
 674 was 0.054, 0.098, 0.093 and 0.091, respectively. The corresponding standard
 675 coefficient was 0.928, 0.246, -0.166 and 0.050 implying that the absolute relative
 676 contribution was 66.8, 17.7, 11.9 and 3.6%, respectively. Thus, TL and T_{VN}
 677 contributed clearly the most ($t = 69.01$) and least ($t = 3.59$) to F. Tolerance was \geq
 678 0.92. Inclusion of ALI as an expression of growth resulted in the same AIC, i.e., its
 679 contribution was barely insignificant ($t = -1.85$, or $p = 0.065$) and therefore excluded.
 680 F for a standard female of 85 cm with LC diameter 750 μm ($F_{85\text{cm}_750\mu\text{m}}$) ranged from
 681 2.3 (1999) to 3.1 millions (2007).

682 The fecundity of each sample could be successfully described ($p < 0.001$) by the
 683 linear combination of body weight ($W_{\text{corrected}}$) and LC diameter: r^2 ranged from about
 684 0.85 (1999) to about 0.94 (early 2006 and 2008). The higher r^2 in the field compared
 685 to the laboratory (Fig. 3) was the result of the longer range in body size of wild
 686 specimens. Mean C_{weight} of a sample effectively predicted mean $F_{5500\text{g}_750\mu\text{m}}$ ($r^2 =$
 687 0.911 , $p < 0.001$), 5500 g corresponding to W_{expected} of a 85 cm fish (Eq. 3).

688 ***Overall oocyte growth rate of the Barents Sea cod***

689 Development in mean LC diameter (LC_{group}) over time as observed in the Andenes
 690 samples agreed largely with experimental, low-temperature results, although giving

691 somewhat lower LC values at a given calendar day (Fig. 10). The proper Fig 10 near here
 692 establishment of the field-based equation was complicated by two outliers ('late' 2006
 693 and 2007) and one case of large leverage ('early' 2006). The statistical use of all field
 694 data showed an oocyte growth rate (R) of 3.45 (SE ± 1.76) $\mu\text{m}\cdot\text{day}^{-1}$ and an intercept
 695 of -214 (SE ± 196) μm ($r^2 = 0.770$, $p = 0.002$). Avoiding the problem of presenting
 696 the year 2006 twice by leaving out late 2006 in the test, gave comparable outputs (R:
 697 3.69 (SE ± 0.59) $\mu\text{m}\cdot\text{day}^{-1}$; intercept: -278 (SE ± 163) μm ; $r^2 = 0.866$, $p < 0.001$).
 698 Based on the data in Fig. 9 the biggest, positive residual in LC_{group} diameter (2007)
 699 was associated with non-feeding larger fish, which apparently had experienced the
 700 highest vitellogenic temperature. The corresponding most negative residual (late
 701 2006) referred to the most actively feeding fish, and were also among the smallest in
 702 size.

703 *Individual oocyte growth rate of the Barents Sea cod*

704 The individual-based study of all standard Andenes samples combined revealed
 705 that only about 5-7% of the total variation in LC diameter residuals could be
 706 explained by the above defined physical descriptors and transect temperature. These
 707 multiple regressions were, however, highly significant ($p < 0.001$), which should be
 708 seen in light of the high number of females examined ($df = 1,445$). Temperature data
 709 (Vardø North, T_{VN}) as such did not meet the t value > 2 requirement ($t = 1.58$), but
 710 clearly so when multiplied with length (TL) ($t = 3.03$) to form an interaction term ($t =$
 711 3.66). The standard coefficients pointed to that $\text{TL}\times T_{\text{VN}}$ was about 1.6 more
 712 influential than C_{weight} ($t = 2.26$). The addition of C_{viscera} resulted in another
 713 significant, but this time negative contribution ($t = -2.96$). Dropping out C_{viscera} , the
 714 comparable contribution by ALI was $t = 1.94$, i.e., very close to being significant ($p =$
 715 0.053).

716 *Size-specific spawning time in the different waters*

717 For the Barents Sea cod only three out of eight annual standard samples showed
718 evidence of any relationship between LC diameter and total length of each individual
719 fish (Table 2). More specifically, 9% of the variation in 2004 could be explained and
720 16-18 % in 2006-2007, otherwise by a few percent at most. The extra sample in early
721 2006 did not demonstrate such a positive relationship. Year 2000, referring to the
722 latest sample taken in the spring (mid March), showed 12 spawners. Spawners were
723 not significantly different in length from prespawners ($p = 0.536$) (Student t-test).
724 Also, the regression behaved similarly ($p = 0.415$) when spawners were included
725 rounding off their LC diameters to 875 μm to avoid getting false relationships driven
726 by large, swelling oocytes. Similarly, inclusion of five spawners in 2007 did not bias
727 the given regression, i.e., gave $p = 0.003$ instead of $p = 0.002$. Replacement of
728 $W_{\text{corrected}}$ with TL throughout the time series gave similar conclusions while age and
729 number of spawning zones did not significantly ($p > 0.05$) influence LC diameter at
730 any point, despite the large spread in observed values (age: 5-13 years (plus one at 18
731 years); spawning zones: 0-6 (plus one at 7 and 12)).

732 Both the North Sea and Irish Sea samples showed examples of individuals lagging
733 behind in maturation but in opposite to the Barents Sea samples these were generally
734 more concentrated towards the lower part of the size range. In the North Sea the
735 observed spawners were significantly larger in size than their accompanying
736 prespawners ($p = 0.003$) (Mann-Whitney test), i.e., typically by 18 cm (Table 2). This
737 was much less clear for the Irish Sea ($p = 0.476$) where the spawners were on average
738 only 3 cm larger (Student t-test). However, in this case a meaningful comparison was
739 complicated because prespawners were also very close to spawning, seen by their
740 large LC_{group} diameter (822 μm) (Table 2). Prior to standardisation using Eqs. (11)

741 and (12), both the North Sea and Irish Sea data showed a significant positive fish size-
742 dependency (North Sea: $r^2 = 0.137$, $df = 1,33$, $p = 0.029$; Irish Sea: $r^2 = 0.238$, $df =$
743 $1,35$, $p = 0.002$). Following standardisation this significance still prevailed (North
744 Sea: $r^2 = 0.152$, $df = 1,39$, $p = 0.012$; Irish Sea: $r^2 = 0.155$, $df = 1,36$, $p = 0.014$),
745 setting, as above, the LC diameter of spawners to 875 μm .

746 **Light cycle in the different waters**

747 The light cycle in the Barents Sea differs markedly from the other areas of interests
748 and is characterised by continuous light during the summer followed by a steep
749 decline in day length to continuous 'darkness' in the winter, i.e., with a much larger
750 amplitude in day length than the Channel, Irish Sea and experimental location (Fig. Fig 11 near here
751 11). These latter three show quite similar light cycles, although following, as
752 expected, the general pattern of a larger temporal range in day length northwards.

753 **Conceptual maturation model**

754 *Approaches taken*

755 The fact that the oocyte growth curves of the low-temperature experimental cod
756 and the Barents Sea cod were close (Fig. 10) despite the great difference in light cycle
757 suggested that autumnal equinox could be a collective starting point for vitellogenesis,
758 simply because this was the only point between summer and winter time when their
759 day lengths were exactly equal (Fig. 11). The inference of an underlying similar
760 oocyte growth pattern following temperature adjustments was strengthened because
761 the LT curve referred to 5 °C (Fig. 5) whilst the Barents Sea cod likely stayed in
762 somewhat cooler water (Figs. 8 and 9); the plotted field curve should be located
763 somewhat below the experimental LT one, as noticed in Fig. 10. Detailed
764 examinations of Eq. (6) and (7) showed that these two experimental curves intersected
765 on Day 130 (8 October) corresponding to a LC_{group} diameter of 250 μm (LT: 249 μm ;

766 AT: 252 μm) followed in both cases by initiation of vitellogenic oocyte growth. This
 767 was seen from the combination of 1) 250 μm is maximum previtellogenic oocyte
 768 diameter, 2) oocyte growth is linear and 3) vitellogenesis is well established on Day
 769 155, each point being described in full above. Thus, the roughly two-week period
 770 from autumnal equinox on Day 114 (22 September) to this intersection point was
 771 defined as response time ('latency'), and the two equations rewritten as:

772

773 (6)' $LC_{\text{group}} = 3.43(\text{SE} \pm 0.20) \times \text{ED}_{\text{vit}} + 250 \text{ (LT)}$

774

775 (7)' $LC_{\text{group}} = 4.10(\text{SE} \pm 0.15) \times \text{ED}_{\text{vit}} + 250 \text{ (AT)},$

776

777 where ED_{vit} is number of days after 8 October (ED_{vit} in the following year will
 778 therefore be 84 + calendar day). In consequence, the problem of locating the intercept
 779 value, known to be important (see above), was assumed solved. A remaining problem
 780 was to clarify the actual vitellogenic temperature ($T_{\text{vit.}}$) of the present Barents Sea cod
 781 bearing in mind that the above data indicated some differences in CTD and DST
 782 temperature records. Accordingly, $T_{\text{vit.}}$ was considered unknown and indicated by
 783 adjusting the oocyte growth rate, R_{new} , in the general equation $LC_{\text{group}} =$
 784 $R_{\text{new}} \times \text{ED}_{\text{vit}} + 250$ to achieve a fit between this curve and the field-based one. This
 785 happened when R_{new} was $3.36 \mu\text{m} \cdot \text{day}^{-1}$ (Fig. 10) reflecting a temperature of $3.4 \text{ }^\circ\text{C}$,
 786 found by rearrangements of Eq. (8). Thus, the Andenes cod apparently stayed mostly
 787 in the upper part of the relevant DST temperature range (Fig. 8), i.e., consistently in
 788 colder water than indicated by the CTD Vardø North Transect (Fig. 9).

789 ***Resulting output***

790 The combined use of Eq. (8) and the expression $ED_{vit} = 625/R_{new}$ made it possible
791 to model the start of spawning (i.e. $LC = 875 \mu\text{m}$) in the year for an individual female
792 cod in response to a range in T_{vit} . (Fig. 12). As can be seen, Eq. (8) was extrapolated
793 by 3-4°C on each side of the range to include higher and lower temperatures than used
794 experimentally and adopting the associated level of uncertainty. Any dependency on
795 fish size was tested in a warm water situation (see above) using Eq. (10) followed by
796 the previous standard procedure setting the temperature to 11 °C, i.e., the typical DST
797 T_{vit} seen for southern waters (Fig. 8). However, the low r^2 of Eq. (10) implied
798 considerable prediction bands (not shown) and thereby gave an uncertain conclusion
799 about the actual levels of response (Fig. 12).

Fig 12 near here

800 ***Realism test***

801 The conceptual model, excluding any body size effects, was tested by consulting
802 published spawning curves (seasonal pelagic egg production curves) of the Barents
803 Sea (Pedersen 1984: Lofoten area) and Irish Sea cod (Armstrong et al. 2001). Thus,
804 start of spawning (egg release) was known meaning that the matching T_{vit} could be
805 found from Fig. 12 and validated with available oceanographic data (Barents Sea cod)
806 or DST information (Irish Sea cod). Resulting vitellogenic temperatures agreed well
807 with expected environmental water temperatures encountered by the fish, however, in
808 the case of the Barents Sea cod these earliest spawners (calendar day 75) likely
809 originated from local waters. More specifically, T_{vit} equaled 7.5 °C corresponding
810 with the typical autumnal temperature of 7 to 8 °C seen in Atlantic water masses (50-
811 200 m) off Lofoten in the Gimsøy Transect (68°24'N 14°04'E –70°24'N 0812'E) (Dr
812 K.A. Mork (personal communication, 2008). In the case of the Irish Sea start of
813 spawning (calendar day 45) referred to a T_{vit} of 13°C, which was possible (Fig. 8).

814

815 **Discussion**

816 Our study on reproductively competent Atlantic cod has revealed the interaction
817 between the main factors influencing the maturity (oocyte growth) dynamics of this
818 species. The strong dependence between day length and the initiation of vitellogenesis
819 enabled us to develop temperature-specific maturity formulae based on general
820 physiological principles, i.e., not requiring any new data to run the model in future
821 operations. We were then able to use these formulae to predict convincingly the
822 variation in start of spawning time in cod of several different stocks. Thus, other
823 factors affecting oocyte growth rate such as condition variation and body size did not
824 significantly bias this calculation. These results now make it possible to better
825 understand the variation in cod spawning time as a consequence of past marine
826 climate, and allow us to make forecasts about what may happen in the future as
827 climate changes.

828 **The effect of the autumnal equinox on maturity**

829 Here we demonstrate experimentally that autumnal equinox is the starting point of
830 vitellogenesis in Atlantic cod. This can be deduced from the fact that the fish in two
831 different tanks were maintained at very different temperatures from summer onwards
832 but did not display any sign of different oocyte growth trajectories until 8 October.
833 The models that we fitted to the data show that the growth curves for oocytes in each
834 experimental tank intersected at 250 μm corresponding to the upper previtellogenic
835 oocyte (PVO) diameter described in other studies (Sivertsen 1935; Kjesbu 1991). This
836 result concurs with Woodhead and Woodhead's (1965) conclusion, based on
837 observation of a concurrent sharp increase in thyroid follicle cell height and increase
838 in size and numbers of late PVOs (circumnuclear phase oocytes), that autumnal
839 equinox is the time of 'spawning migration and gonad maturation' for the Barents Sea

840 cod. Previously, Woodhead and Woodhead's work has received little attention,
841 possibly because the spawning migration of the Barents Sea cod starts much later, i.e.,
842 in December-January (Bergstad et al. 1987), and so is easily disassociated from gonad
843 maturation in this stock. However, the regulation of thyroid hormone production was
844 further explored by Comeau et al. (2001) who observed a significant increase in these
845 hormones around the equinox in the southern Gulf of St. Lawrence cod. Their results
846 on estradiol-17 β and testosterone show a (minor) pulse around that time. Surprisingly,
847 sentinel catches in the western part of this gulf area consistently peaked on exactly the
848 same date as the present oocyte growth intersection point, 8 October (2-13 October)
849 followed by another catch peak about two weeks later in the eastern part of the gulf
850 when the migrating cod arrived. Comeau et al. (2001) conclude that 'thyroid
851 hormones may facilitate the onset of the autumn migration by enhancing metabolism,
852 sensory biology and swimming capacity'. Although it is new information that
853 vitellogenic oocyte growth in cod typically commences after a latency of about two
854 weeks following autumnal equinox, studies related to cod aquaculture, especially on
855 photoperiod manipulation, agree with our conclusion as these report increased levels
856 of sex steroids and gonad growth from October onwards in the normal day group
857 (Norberg et al. 2004; Skjæraasen et al. 2004; Davie et al. 2007). Such experimental
858 designs also reflect that the oocyte growth rate can show a great level of plasticity
859 (Hansen et al. 2001; Davie et al. 2007), but in a field situation the photoperiodicity
860 should be considered constant between years, ignoring the possible effect of variation
861 in cloud covers. Our study is exceptional in that it finds an almost perfect matching of
862 laboratory and field data on the temperature dependence of oocyte growth, which 1)
863 emphasizes the success of our experimental design and the accuracy of both the

864 experimental and field datasets and 2) opens the road for similar experiments to take
865 place for other fish species.

866 Present results showed that the experimental cod lost their appetite and grew less
867 between 6 October and 2 November. Skjæraasen et al. (2004) observed a similar
868 sudden drop in food intake for cod held on natural light and considered the following
869 decline in appetite up to spawning to be a consequence of sexual maturation, although
870 information shows that cod may take food during spawning (Fordham and Trippel
871 1999; Michalsen et al. 2008). Hence, the time around autumnal equinox is obviously a
872 period where major changes take place in the physiology of adult cod, including a
873 switch in energy allocation patterns to support further gonad growth. The
874 experimentally delayed and compressed seasonal photoperiod data in Norberg et al.
875 (2004) show that testosterone for both female and male cod consistently increases
876 when the day length falls below 12 hours. For females, the corresponding estradiol-
877 17β pattern lags slightly behind, which is as expected; testosterone is 'aromatased'
878 into estradiol- 17β (see Norberg et al. 2004). Thus, it is not the calendar as such that
879 determines the onset of maturity, but the time when the duration of darkness first
880 exceeds 12 hrs in the autumn, presumably through the mechanism of melatonin
881 accumulation (Migaud et al. 2007). The present outlined day length threshold value
882 and subsequent vitellogenic response is remarkably similar to mechanisms
883 demonstrated in the marine annelid *Nereis virens* (Olive et al. 1998).

884 The experimental oocyte growth data do, however, show a variation in initiation of
885 vitellogenesis of about ± 1 month. Thus, a few individuals start to show evidence of
886 oocyte growth in September while others start in November, corresponding to a day
887 length of 14 and 10 h, respectively. Consequently, early spawners (those that require
888 only 10 h of darkness to start oocyte growth) should be considered to be less sensitive

889 to light than late spawners (14 h of darkness). One applied consequence of this
890 discovery is that considerable savings could likely be made in the cod aquaculture
891 industry by replacing the current practise of continuous light (24 h light: 0 h dark) to
892 prevent sexual maturation (Taranger et al. 2006) to obviously somewhat shorter day
893 lengths to reduce the electricity bill.

894 **The effect of temperature on reproductive investment and condition factor**

895 We found ample experimental evidence of an influence of temperature on
896 reproductive investment, temporal variation in body growth and condition factor.
897 Early on in the experiment, females held at the higher, ambient temperature (AT)
898 showed indications of better appetite, compared to females held at the lower, cooled
899 temperature (LT), although the overall feeding ration for the whole length of the
900 experiment was not statistically different. In both treatments, oocyte recruitment
901 ended during the late autumn, with a few exceptions, as is usually observed for
902 determinate spawners such as cod (Kjesbu 2009). However, despite indications of a
903 higher food intake, AT females had a lower condition index (Fulton's K) than LT
904 females. This is in contrast to previous field studies across cod stocks that report
905 higher K at higher temperatures (Rätz and Lloret 2003). In our experiment the main
906 reason appears to be generally more investment in length growth at AT but similar
907 overall weight growth as LT putting the estimated K for AT downwards. This finding
908 was further supported by that 1) tests on somatic K, i.e., following subtraction of
909 ovary weight from the expression, also gave a significant difference and 2) these
910 differences in K were already in place in early October, i.e., before vitellogenesis and
911 thereby gonad growth was well established. Regression analyses standardised for
912 maturity demonstrated that differences in relative fecundity between the two
913 categories of females was established early on (LC diameter \approx 400 μ m) pointing to an

914 increased production of PVOs at AT compared to at LT. The noted higher fecundity
915 at AT apparently came at the expense of reduced liver size. In contrast, LT females
916 appeared to ‘over-recruit’ oocytes, and later significantly reduced the number of
917 developing oocytes as vitellogenesis (LC diameter) progressed, a process known as
918 down regulation through vitellogenic atresia (Witthames et al. 2009). These different
919 patterns might suggest that the AT regime is more ‘effective’ in terms of oocyte
920 recruitment, i.e., assumed to be closer to the upper pejus temperature (Pörtner et al.
921 2001; 2008): a vitellogenic temperature of 9 °C enabled the AT females to reduce
922 investment in the liver to boost egg production, whereas the LT group retained
923 investment in the liver at a less ‘optimal’ temperature of 5°C. Taken together these
924 results show that interpretation of K data on cod (for example from field surveys)
925 require a good understanding of feeding conditions as well as thermal experience, and
926 therefore such data should be treated with caution. Conversely, our findings support
927 the conclusion of Skjæraasen et al. (2006) that the period of early vitellogenesis is
928 important for the resulting fecundity of cod but here we clarify that the underlying
929 oocyte regulation pattern depends on temperature and thereby varies with temperature
930 and can be traced back as early as summer time.

931 **The effect of temperature and other factors on oocyte and ovary growth**

932 Unlike previous work (Kjesbu 1989) to estimate Q_{10} , our experiments were
933 conducted during the vitellogenic period rather than the spawning season. The rates of
934 growth of oocytes, and thereby the corresponding Q_{10} values, are therefore lower
935 because the estimates of growth were not made during the period when oocytes swell
936 with water (Kjesbu et al. 1996). As a result, our experiments enabled us for the first
937 time to establish a robust relationship between the rate of oocyte growth and

938 temperature during the full maturity period, paying special attention to the assessment
939 of the Q_{10} value and its underlying rate-specific error terms.

940 In any experiment of this nature, it is not possible or desirable to totally negate
941 uncontrolled effects on oocyte growth created e.g. by variation in fish condition
942 (Kjesbu 2009) or potential trade-off with somatic growth (Yoneda and Wright 2005).
943 However, the modelled specific growth rates (G) of cod (of the size we used in our
944 experimental tanks) are almost constant over the temperature ranges we selected (as
945 per Bjørnsson and Steinarsson 2002) and should cancel out any overall difference in
946 body growth, as we found. More specifically, the present overall G values of LT and
947 AT fell between two earlier published records, one from the laboratory (Bjørnsson
948 and Steinarsson 2002: Icelandic cod), which was 17 and 9% above, and one from the
949 field (Clark et al. 2003: North Sea), which was 15 and 20% below, respectively.
950 Similarly, the applied *ad libitum* feeding protocol should, in theory, remove any
951 general condition effect as such. Thus, the measurements of leading cohort (LC)
952 diameter over time at 5 and 9°C can be considered to very much reflect the actual,
953 typical effect of these two temperature regimes on oocyte growth. Our experiments
954 therefore provide us with overall oocyte growth rates that were, as far as is possible,
955 solely related to temperature.

956 In consequence, we were able to derive accurate models for maturity (expressed as
957 LC diameter growth) at standard (5-9 °C) and extrapolated (2-13°C) temperatures.
958 These did not reveal any deviation from the observed spawning curves in the northern
959 or southern stocks (as derived from field sampling). In addition, the temperature data
960 collected from the electronic tagging experiments showed that the temperatures at the
961 upper limit of this extrapolation can be considered normal for wild cod at the southern
962 end of their distribution. Thus it seems likely that changes in spawning time, based on

963 the maturity curves, can be predicted at these higher temperatures. We are, however,
964 more uncertain about the validity of this model at the other end of the temperature
965 scale (from 2 to 5°C) because the directly measured thermal experience of cod in the
966 southern stocks has rarely fallen this low (Neat and Righton 2007, D. Righton
967 (personal communication, 2009)). Historical data on water temperatures at the
968 southern limits of cod distribution suggest that such circumstances have occurred in
969 the past (Bigg et al. 2008).

970 One potentially important problem not accounted for in the present maturity
971 models is that mature fish in poor condition are known to delay spawning time up to
972 two weeks (see review in Kjesbu 2009). This length of time compares with what was
973 seen in the Andenes time series in terms of total residual variation in LC_{group} diameter
974 (when considering the relevant oocyte growth rate). Only 5-7 % of this variation
975 could, however, be explained by the set of selected predictors in this work including
976 relative condition. This result was markedly different from the corresponding
977 fecundity model where almost all of the variation, 92%, could be explained by the
978 same predictors. In other words, fecundity and spawning time show fundamentally
979 different main regulatory principles, as implied in the above discussion on the
980 dominating role of day light for subsequent spawning time. Other candidates for
981 creating bias when running the present oocyte growth models might be any additional
982 effect of age (Ramsay and Witthames 1996: Dover sole (*Solea solea*) from the
983 English Channel) or spawning experience. These single effects could not be assessed
984 in the experiment because all fish had the same origin. However, the Andenes cod
985 showed large variations in these parametric values but no trace of any relevant
986 implications. Nevertheless, to reduce any uncertainty in the application of the present
987 maturity equations this potential age effect should be further explored, at least in

988 warmer water. Note here that we did find a temperature-specific effect of body size,
989 dealt with later on. Further tests on the maximum vitellogenic LC diameter in relation
990 to fish size (Ramsay and Witthames 1996), presently set to be 875 μm , should also be
991 considered for fine-tuning of the present model.

992 **The effect of temperature on the timing of spawning**

993 Validations showed that our models of oocyte growth were able to predict
994 convincingly the variation in start of spawning time of northern and southern cod
995 stocks in the north-east Atlantic. Thus, spawning time in the southern stocks always
996 occurred earlier than in the northern stocks and, because the variation in thermal
997 experience during vitellogenesis was greater to the south, spawning time was more
998 variable than in the north. Furthermore, our model can help to explain variation in
999 spawning time for stocks outside of our study region. For example, the observed
1000 water temperature and spawning time information presented for cod off
1001 Newfoundland (Hutchings and Myers 1994) also agree well with our models despite
1002 very low ambient temperatures and a different definition of ‘time of spawning’.
1003 However, for unknown reasons, our model does not seem suitable for the Eastern
1004 Baltic Sea cod, which may also spawn in the early autumn even though the
1005 experienced temperatures do not seem to be particularly cold (Wieland et al. 2000).
1006 This situation might be explained because the long Baltic spawning season (March-
1007 September) is a special adaptation to the extreme fluctuating environmental
1008 conditions in this ecosystem (MacKenzie et al. 1996). Also the Baltic tribe is an
1009 outlier both physiologically (in terms of egg formation) and genetically (Kjesbu and
1010 Witthames 2007) as well as by its more pelagic life style than typical for adult cod
1011 (Tomkiewicz et al. 1998).

1012 **Understanding the spawning dynamics of cod stocks**

1013 In most areas, the progression of the spawning season in cod is observed as a
1014 gradual and accelerating increase in egg density to a peak, and a subsequent
1015 deceleration (the ‘spawning curve’). The experiments we conducted show that the
1016 start of vitellogenesis is imprinted (Otterå et al. 2006; Greives et al. 2008; Paul et al.
1017 2008) but the subsequent oocyte growth rate is adjusted by environmental temperature
1018 (Olive et al. 1998). Thus, autumnal equinox acts as the oocyte growth trigger and
1019 temperature as the main oocyte growth regulator. These results lead to a new
1020 perspective of the principles involved in the formation of what appears as this typical
1021 spawning curve, as seen for instance in the Lofoten area (Pedersen 1984). The first
1022 part of this curve should consist of eggs shed by fish coming from the warmest water
1023 followed progressively by fish coming from colder and colder water. In the case of
1024 Lofoten this would indicate that local cod (i.e. Coastal cod) eggs are generally
1025 spawned first followed later also by eggs from the Barents Sea cod.

1026 Secondly, our data support the contention that the spawning time of larger females
1027 in relation to smaller females is advanced in the warmer water but this phenomenon
1028 vanishes in the colder water. The underlying reasons for size dependency are,
1029 however, unclear (Wright and Trippel 2009). According to Pörter et al. (2008), a
1030 larger ectothermic body enhances thermal sensitivity based on allometrical
1031 considerations: ‘oxygen supply becomes restricted earlier than in a smaller specimen’.
1032 For the Barents Sea cod the size dependency seems labile; in a few years larger cod
1033 are more developed, in most other years, they are not. For the Irish Sea and North Sea
1034 both data sets indicate that the larger cod spawn first, although less convincing for the
1035 Irish Sea than the North Sea, possibly due to the less successful sampling program but
1036 also truncated age and length distribution following the stock collapse in the Irish Sea
1037 stock. Taken together, this suggests that size-specific spawning time apparently has an

1038 underlying physiological reason related to thermal window dynamics, found
1039 experimentally to relate back to body size in summer time at AT. In this article, the
1040 information from DSTs suggests that the spawning temperature (window) is around 7
1041 °C for cod in southern waters, the Irish and North Seas and the Channel, and around 4
1042 °C for cod in northern waters, the Barents Sea. Thus, the two spawner categories seem
1043 adapted to different thermal windows, as expected (Pörtner et al. 2008). More data
1044 are, however, needed to stretch this argument any further, e.g. earlier findings suggest
1045 that the Barents Sea cod may spawn between 4 and 6 °C (Ellertsen et al. 1989).

1046 **Predicting the effects of climate change on cod reproductive ecology**

1047 In sum, the results of our work show that we are now able to explain and predict
1048 the maturity (oocyte growth) and likely fecundity and reproductive success of cod in
1049 different areas. The tool box of equations that we derived should now make it possible
1050 to better understand the variation in cod spawning time as a consequence of future
1051 climate change that, in turn, will have great prospects in further recruitment studies.
1052 For instance, one could now examine if the effects of climate-mediated changes in the
1053 zooplankton community (e.g. Beaugrand et al. 2000), coupled with changes in the
1054 time of spawning, could lead to a more frequent mismatch between the critical
1055 feeding period for cod larvae and the time of greatest abundance of copepod nauplii.
1056 Also, the consequences of warming on the success of ovulation and egg quality in
1057 different waters should be further examined paying special attention to the critical
1058 threshold value of 9.6 °C for cod seen in the aquaculture-related work of van der
1059 Meeren and Ivannikov (2006).

1060 Conversely, temperatures suitable for optimal vitellogenesis may be actively
1061 selected by adult cod, assuming that these temperatures also allow for sufficient food
1062 intake to permit sufficient investment in the gonads, and therefore mediate the effect

1063 of any climate change by shifting the relative positions of feeding and spawning
1064 habitats. Turning the present series of arguments around, our findings may suggest
1065 that observations of spawning curves can be used to indicate the temperatures that
1066 adult cod have been experiencing, i.e., the ovaries of cod could be used as a
1067 ‘biological thermometer’.

1068 Altogether, we show that the underlying oocyte growth and energy allocation
1069 patterns of cod is strongly influenced by environmental temperature opening up for a
1070 fascinating field of research, and that the issues discussed in this paragraph and the
1071 others above will be central research areas for marine fish reproductive physiologists
1072 in the years to come.

1073

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1089

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Table 1. Summary of experimental results on female Coastal cod held at low temperature (LT) and ambient temperature (AT) during their second maturation cycle, i.e., from the age of about 2½ to 3 years. In one case both genders were analysed for comparison with published results. Prespawner and spawner refer to females that became these categories towards the end of the experiment (Day 224). **Note:** W, whole body weight; TL, total length; G, specific growth rate; DLI, daily length increment; K, Fulton's condition factor; GSI, gonadosomatic index; HSI, hepatosomatic index; F, fecundity; RF, relative fecundity; n, number of fish.

	Category	No. of days	LT		AT		Between-tank (P-value)
			n	Mean (SD)	n	Mean (SD)	
Growth							
W (g)	Female	0	33	1861 (298)	38	1866 (350)	0.955
	Female	224	33	3348 (482)	38	3469 (589)	0.351
TL (cm)	Female	0	33	53.6 (3.4)	38	54.4 (3.2)	0.317
	Female	224	33	61.5 (3.8)	38	64.2 (3.9)	0.004
G (%•day ⁻¹)	Both genders	224	75	0.252 (0.052)	74	0.253 (0.059)	0.624
	Female	224	33	0.263 (0.036)	38	0.278 (0.045)	0.086
DLI (mm•day ⁻¹)	Female	224	33	0.35 (0.05)	38	0.44 (0.08)	< 0.001
Reproduction							
W (g)	Female, prespawner	0	31	1855 (306)	25	1774 (304)	0.328
	Female, spawner	0	2	1961 (–)	13	2042 (377)	—
	Female, prespawner	224	31	3354 (496)	25	3340 (535)	0.921
	Female, spawner	224	2	3257 (–)	13	3717 (629)	—
K	Female, prespawner	224	31	1.42 (0.15)	25	1.28 (0.15)	0.001
	Female, spawner	224	2	1.68 (–)	13	1.34 (0.11)	—
GSI (%)	Female, prespawner	240	30	11.8 (3.0)	25	14.1 (3.7)	0.013
	Female, spawner	240	2	15.7 (–)	13	22.2 (4.1)	—
HSI (%)	Female, prespawner	240	31	13.2 (1.5)	25	11.0 (1.2)	< 0.001
	Female, spawner	240	2	12.7 (–)	13	10.1 (1.4)	—
F (millions)	Female, prespawner	240	30	3.72×10 ⁶ (0.70×10 ⁶)	25	4.32×10 ⁶ (1.02×10 ⁶)	0.017
RF (g ⁻¹)	Female, prespawner	240	30	1117 (167)	25	1307 (242)	0.003

1313

Table 2. Overview of samples, split into prespawners and spawners, used to explore the relationship between leading cohort (LC) oocyte diameter and total length (TL) in the different waters of study. For the Barents Sea cod year-specific regression were established. If significant, slope (a) and intercept (b) values (\pm SE) are given. In 2006 both an early and late sample was taken. North Sea and Irish Sea LC diameter data were standardised to the last day of sampling at the cruise. Shaded area: a significant result; solid border: an insignificant result (for TL only). n: number of females.

Year	Area	Calendar day	n	Category	TL (cm)		LC (μ m)		Regression analysis LC vs. TL			
					Mean	SD	Grand mean	Grand mean SD	r ²	P	a (\pm SE)	b (\pm SE)
<i>Northern area</i>												
							<i>Observed values</i>					
1999	Barents Sea	69-70	90	Prespawner	85.2	9.7	752	62	0.001	0.758	–	–
2000	Barents Sea	74	79	Prespawner	80.8	9.4	792	64	0.007	0.456	–	–
2003	Barents Sea	62	48	Prespawner	84.9	14.7	732	64	0.002	0.793	–	–
2004	Barents Sea	57	51	Prespawner	86.1	16.1	722	52	0.086	0.037	0.95 (0.44)	640 (39)
2005	Barents Sea	59	45	Prespawner	89.7	13.4	717	65	0.038	0.199	–	–
2006, early	Barents Sea	43-44	38	Prespawner	90.5	14.8	680	76	0.001	0.825	–	–
2006, late	Barents Sea	66-67	40	Prespawner	82.1	17.9	724	59	0.158	(0.011)	1.31 (0.49)	613 (54)
2007	Barents Sea	67	49	Prespawner	91.3	17.8	785	79	0.180	0.002	1.88 (0.58)	613 (54)
2008	Barents Sea	58-59	46	Prespawner	92.8	14.7	725	64	0.004	0.687	–	–
<i>Southern area</i>												
							<i>Standardised values</i>					
2004	North Sea	22-55	17	Prespawner	60.9	16.1	706	94				
			24	Spawner	78.9	15.3		\geq 875				
2004	Irish Sea	41-50	15	Prespawner	62.7	14.6	822	44				
			23	Spawner	65.9	12.4		\geq 875				

1314

1315

1316 **Figures**

1317

1318 **Fig. 1.** Experimental conditions in low-temperature (LT, filled circles) and ambient-
 1319 temperature (AT, open circles) tanks as described by water temperature (T) (a),
 1320 feeding ration (FR) (b), and resulting mean specific growth rate (G) (\pm SE) (c) and
 1321 mean Fulton's condition factor (K) (\pm SE) (d). Vitellogenesis (shaded area) refers to
 1322 females with developing oocytes. Inserted numbers in (a) are mean vitellogenic
 1323 temperature at LT (lower position) and AT (upper position) while arrow reflects a
 1324 transient problem with the temperature cooler. Solid and broken lines in (b) show
 1325 weighted mean FR at LT and AT, respectively, while overlapping lines in (c) show
 1326 mean G for the whole experiment in the two tanks. Day 0 = 1 June 2005; Day 240 =
 1327 26 January 2006.

1328

1329 **Fig. 2.** Relative somatic fecundity (RF_S) versus leading cohort (LC) oocyte diameter
 1330 on Day 240 (experimental end) for prespawners held either at low temperature (LT)
 1331 or at ambient temperature (AT). LT prespawner is represented by filled circle and AT
 1332 prespawner by open circle. Separate regression lines are included (LT: solid line; AT:
 1333 broken line), excluding in one case an outlier (arrow).

1334

1335 **Fig. 3.** Explanatory power (r^2) of potential fecundity on Day 240 (experimental end)
 1336 for prespawners at low (LT, solid line) or ambient temperature (AT, broken line)
 1337 regressed on either body weight (W) or combined with leading cohort (LC) diameter
 1338 (W + LC) at different measurement points during the experiment. Mean LC diameter
 1339 is attached to each relevant analysis point. W-based regressions are indicated by filled

1340 circle for LT and open circle for AT while W+LC regressions are indicated by filled
1341 and open crosses, respectively. An AT outlier was excluded (see Fig. 2).

1342

1343 **Fig. 4.** Development in mean values (\pm SE) of the width of the developing oocyte
1344 frequency distribution (SD_{diam}) (a) and the diameter of the smallest cohort of oocytes
1345 (SC) (b) in low-temperature prespawners (solid circle) and ambient-temperature
1346 prespawners (open circle) and spawners (cross) between Day 155 and 240 (2
1347 November – 26 January). In b) the vertical distance from maximum previtellogenic
1348 oocyte diameter (horizontal line) to each measurement point indicates oocyte gap size
1349 formation.

1350

1351 **Fig. 5.** Growth in mean leading cohort (LC) oocyte diameter (\pm SE) at the group level
1352 (LC_{group} diameter) at low (filled circle) and ambient temperature (open circle)
1353 between Day 128 and 240 (6 October – 26 January). The same females were tracked
1354 over time.

1355

1356 **Fig. 6** Individual oocyte growth rate (R) between Day 155 and 224 (2 November – 10
1357 January) at low (LT) (filled circle) and ambient temperature (AT) (open circle) in
1358 relation to total length (TL) as measured on Day 0 (1 June). Associated regression
1359 lines (LT: solid line; AT: broken line) are included but excluding in the case of LT an
1360 outlier (arrow).

1361

1362 **Fig. 7** DST-recorded temperatures (T) (monthly mean \pm SD) in released-and-
1363 recaptured individual cod in southern waters, i.e. the Channel (cross), southern North
1364 Sea (square) and Irish Sea (triangle), and Barents Sea (circle). Length of spawning

1365 season in southern (left box) and northern waters (right box) is from Brander (1994)
1366 and Pedersen (1984), respectively. Temperatures at or above the horizontal line (9.6
1367 °C) is considered to result in reduced egg fertilization and normal development (van
1368 der Meeren and Ivannikov 2006).

1369

1370 **Fig. 8** DST-recorded temperatures (mean, min. and max. value) between 1 September
1371 and 1 February for cod in northern and southern areas in relation to total length (TL)
1372 at release (same material as in Fig. 7). The selected period of time is assumed to
1373 encompass the major part of the vitellogenic period and thereby vitellogenic
1374 temperature (T_{vit}), see main text. Cross, square, triangle and circle represent the
1375 Channel, North Sea, Irish Sea and Barents Sea, respectively.

1376

1377 **Fig. 9** Descriptors (mean \pm SD) of the Andenes-caught Barents Sea cod females, i.e.,
1378 total length (TL) (a), viscera condition ($C_{viscera}$) (b) and body condition (C_{weight}) (c)
1379 using filled circle for standard sample and open circle for extra sample, and CTD
1380 temperature (mean only) in the Vardø North Transect in August-September (T_{VN})
1381 (early vitellogenesis) (d). Extreme values in (b) refer mainly to predation on adult
1382 capelin (*Mallotus villosus villosus*) but also adult herring (*Clupea harengus*) and
1383 unidentified 'fish species'. Horizontal line in (b) and (c) is the normalised, reference
1384 line.

1385

1386 **Fig. 10** Grand mean leading cohort oocyte diameter (LC_{group}) as observed in the
1387 Andenes sampling program on Barents Sea cod (filled circle with year attached)
1388 plotted versus calendar day of sampling (solid line) in comparison with similar
1389 experimental data at 5 (short dash, cf. Eq. (6)') and 9.6 °C (long dash, cf. Eq. (7)')

1390 and conceptually modelled data (3.4 °C) (dotted line). Shaded area refers to oocyte
1391 final maturation.

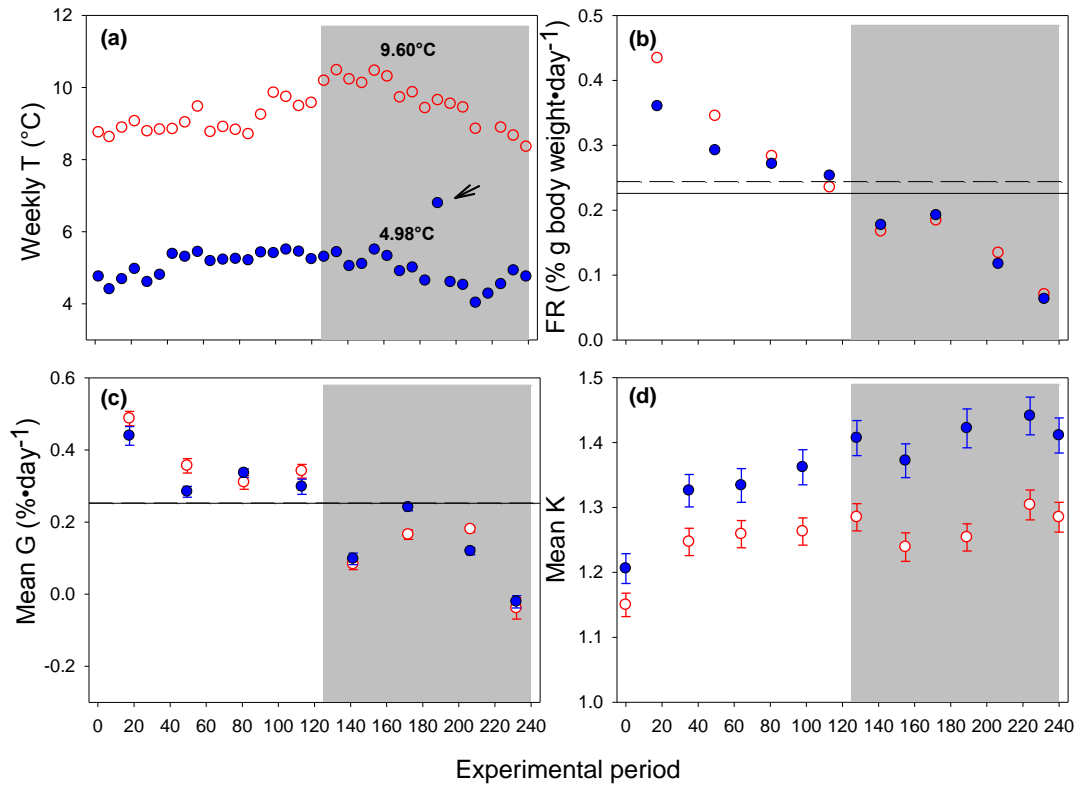
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1393 **Fig. 11** Duration of daylight at four areas of study (Channel: cross; Irish Sea: triangle;
1394 Experiment: square; Barents Sea: circle) as reported by official US web pages at
1395 present experimental measurement days (Day 0-240: 1 June 2005 – 26 January 2006).
1396 Time of summer and winter solstice and autumnal equinox are added as well as the
1397 time when the sun went below the horizon for the first time after the period of
1398 continuous light in the Barents Sea. Horizontal line is inserted to mark autumnal
1399 equinox.

1400

1401 **Fig. 12** Conceptual modelled relationship ($\pm 2 \times \text{SE}$) between start of spawning (i.e.,
1402 leading cohort oocyte diameter equals 875 μm) and vitellogenic temperature (T_{vit}) for
1403 an individual cod female. Spawning time of a large (65 cm, large circle) and small
1404 (55 cm, small circle) warm-water (southern) cod is indicated.

1405



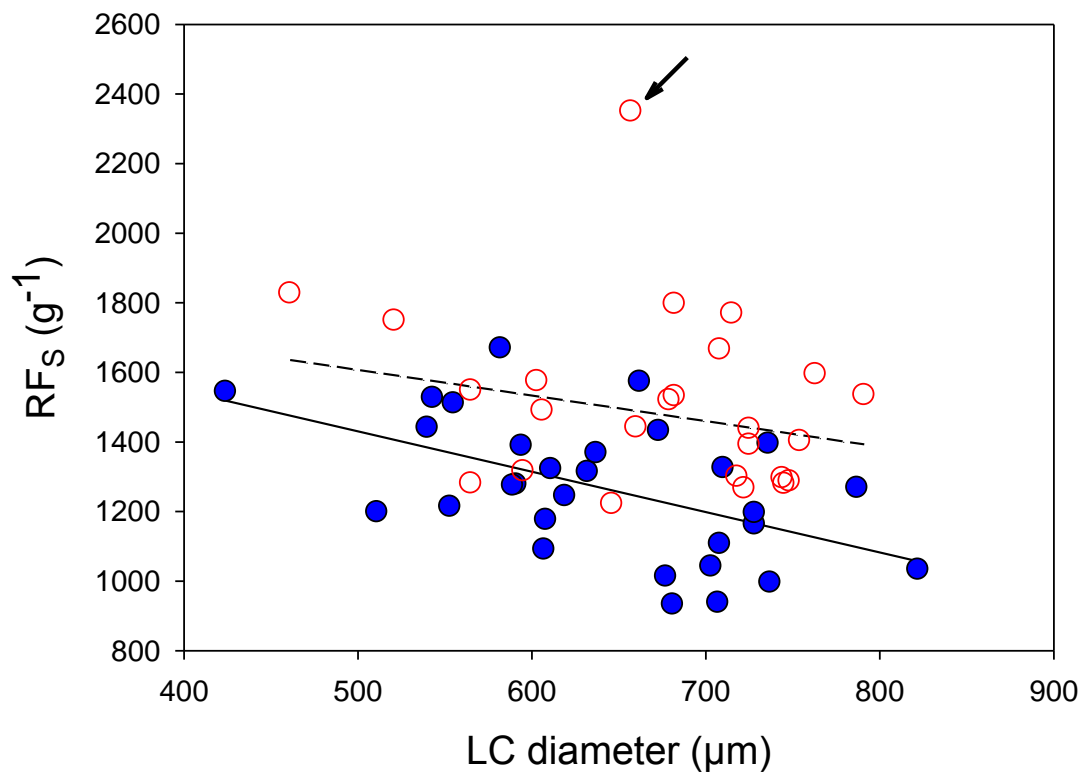
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1407 **Fig. 1**

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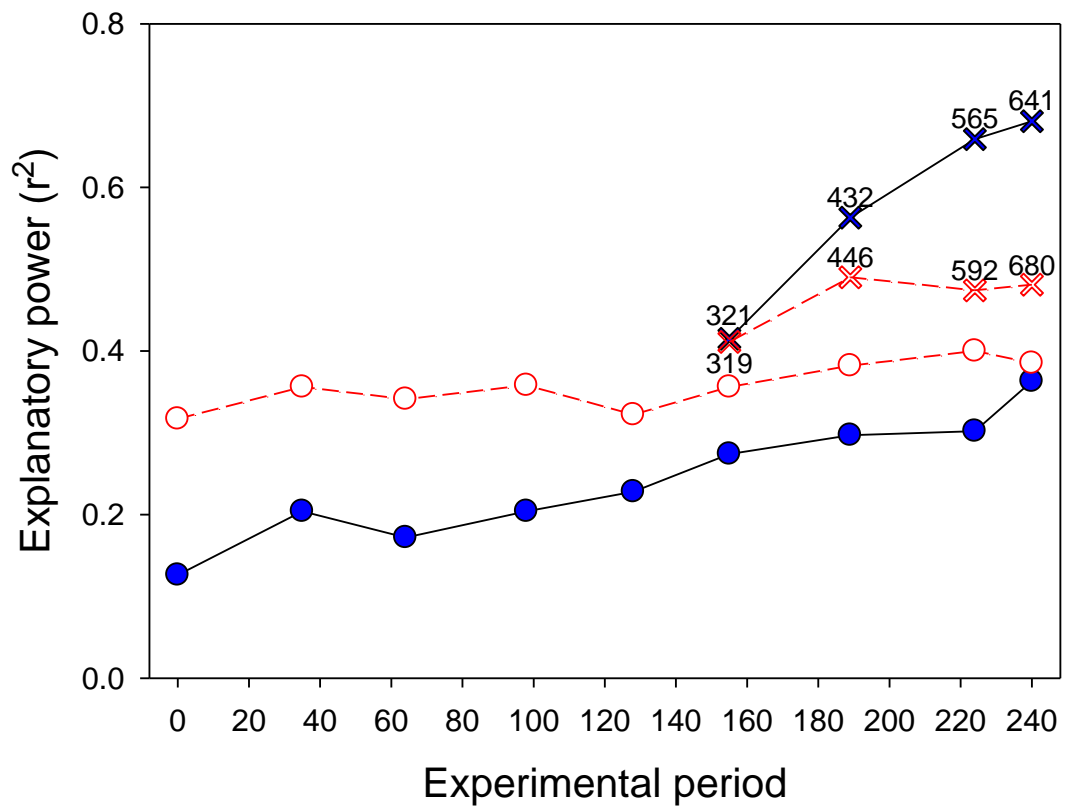


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1412 **Fig. 2**

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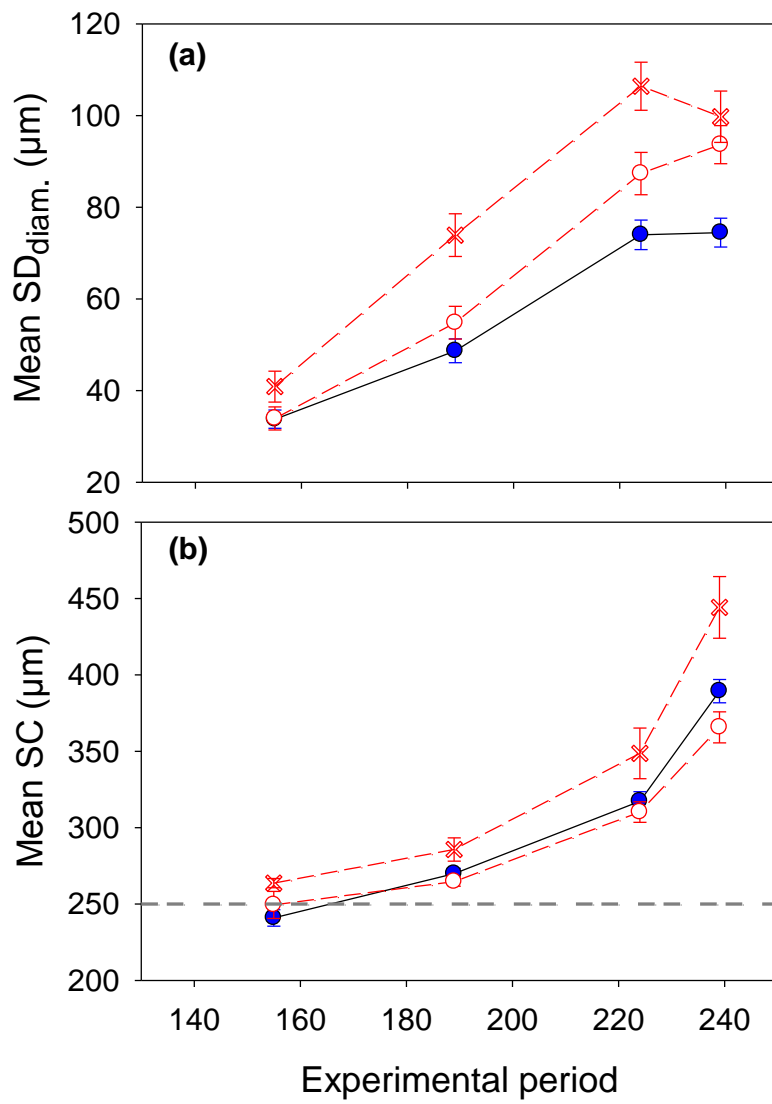


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1416 **Fig. 3**

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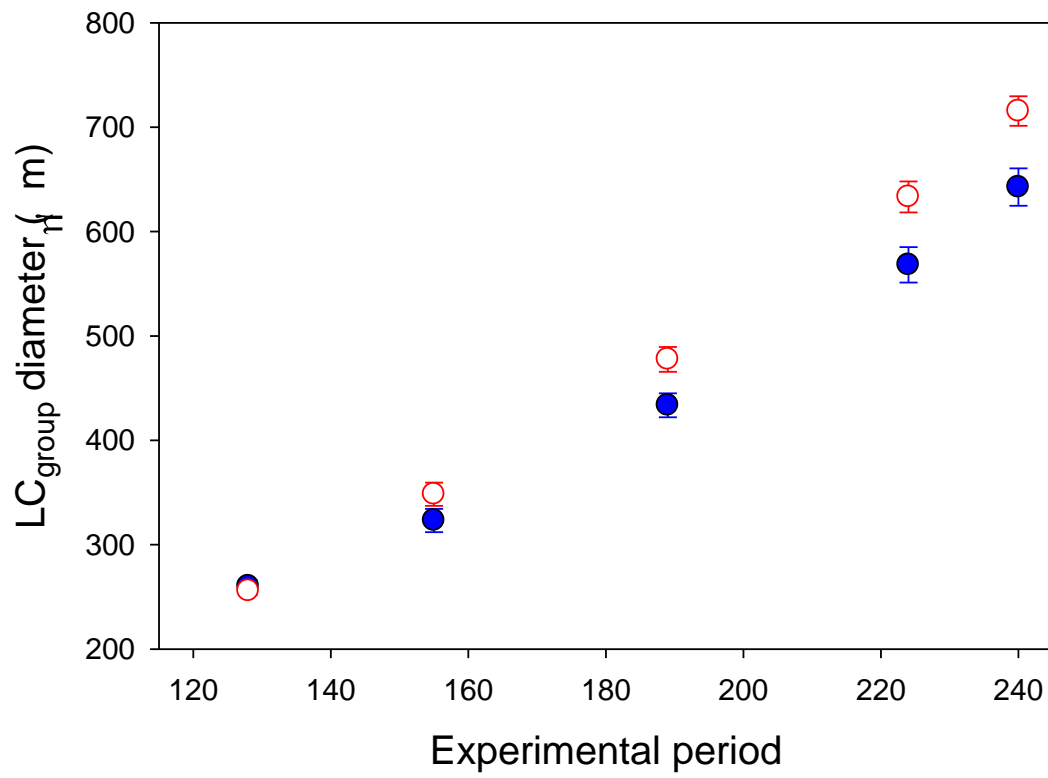


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1420 **Fig. 4**

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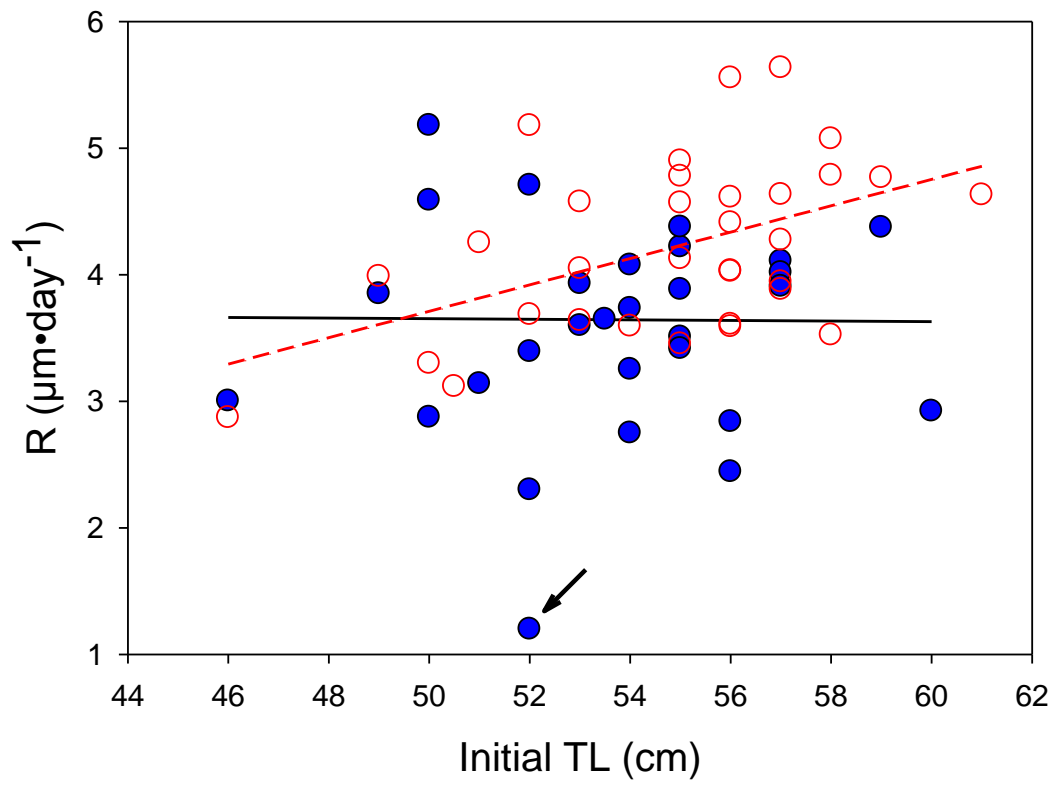
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1424 **Fig. 5**

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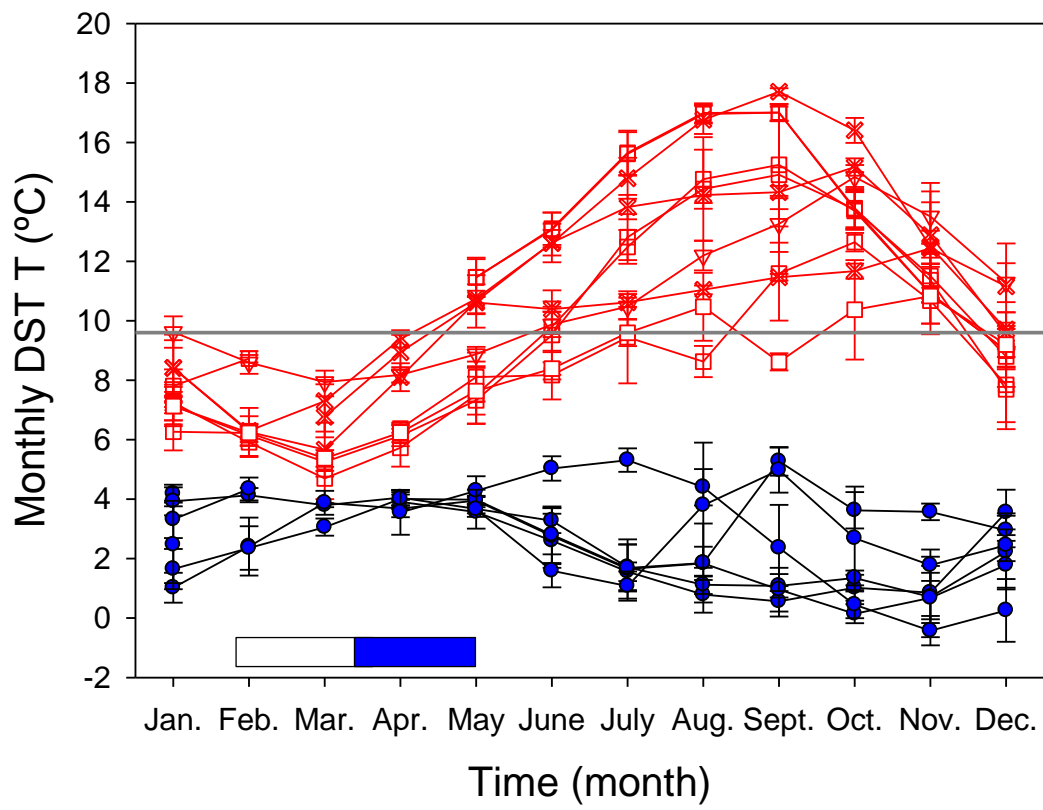


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1429 **Fig. 6**

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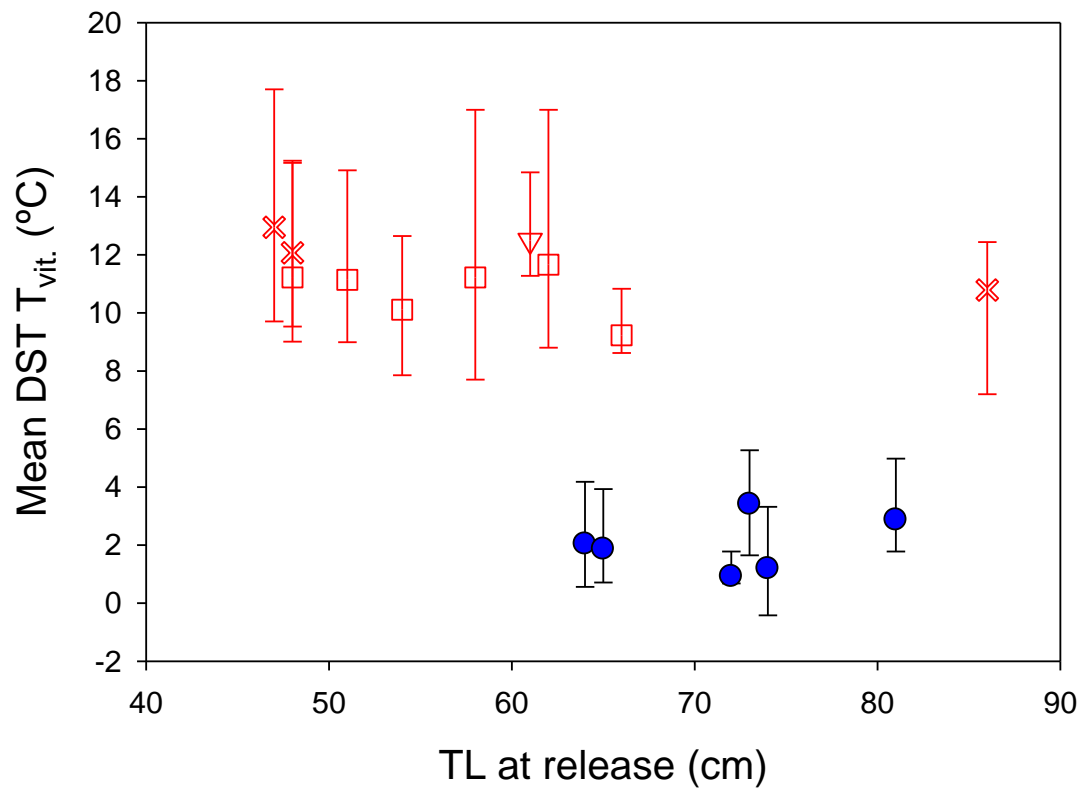


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1433 **Fig. 7**

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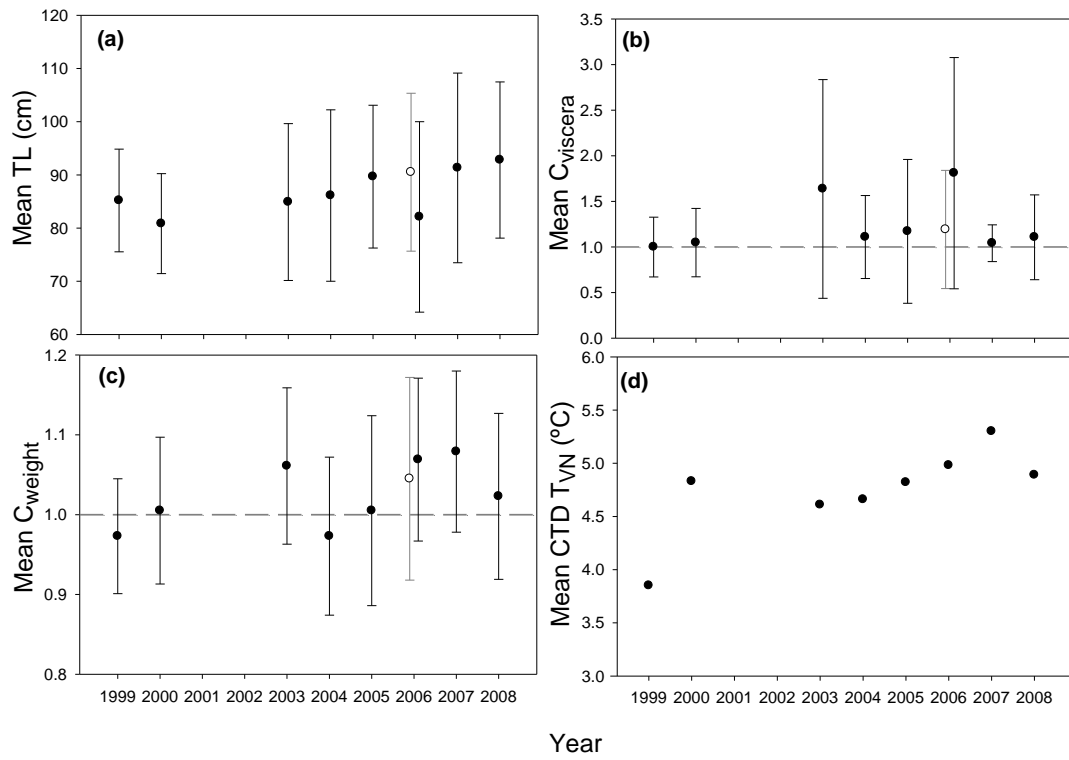


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1437 **Fig. 8**

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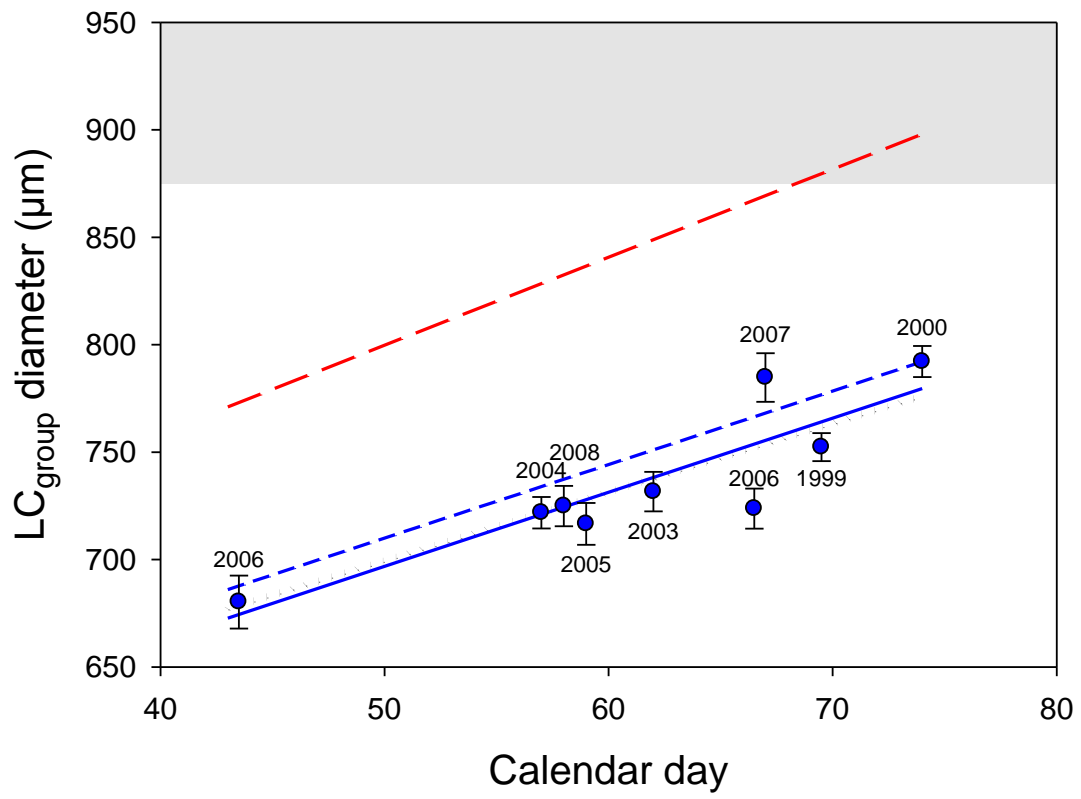


1440

1441 **Fig. 9**

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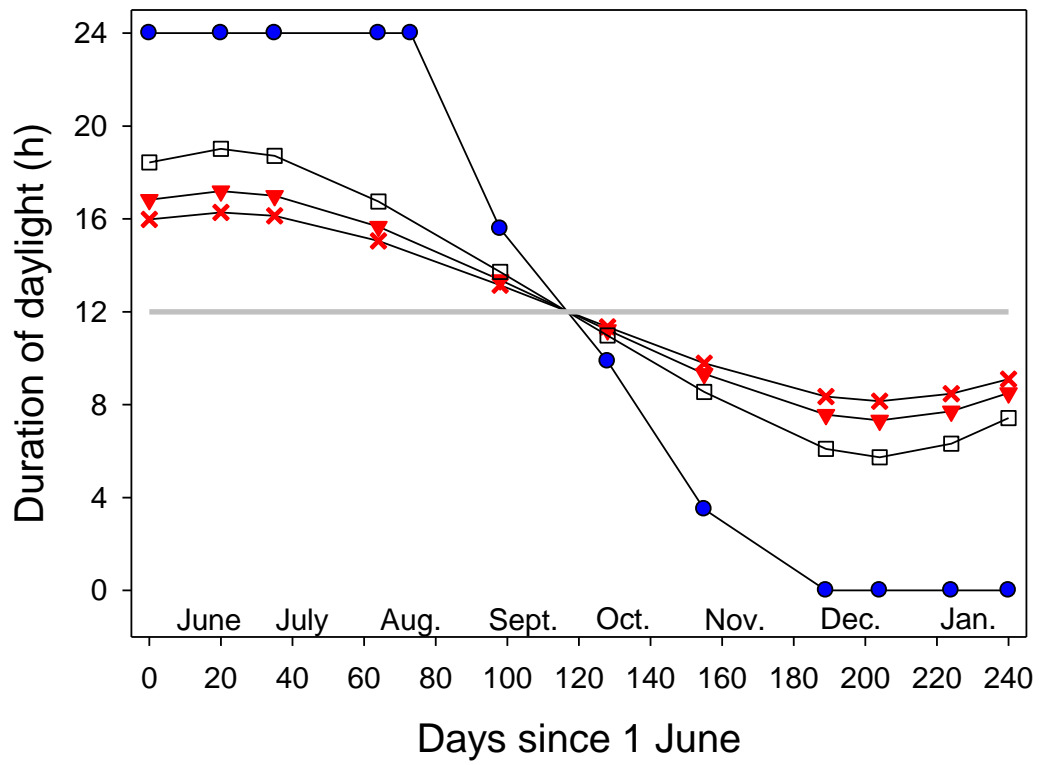
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1445 **Fig. 10**

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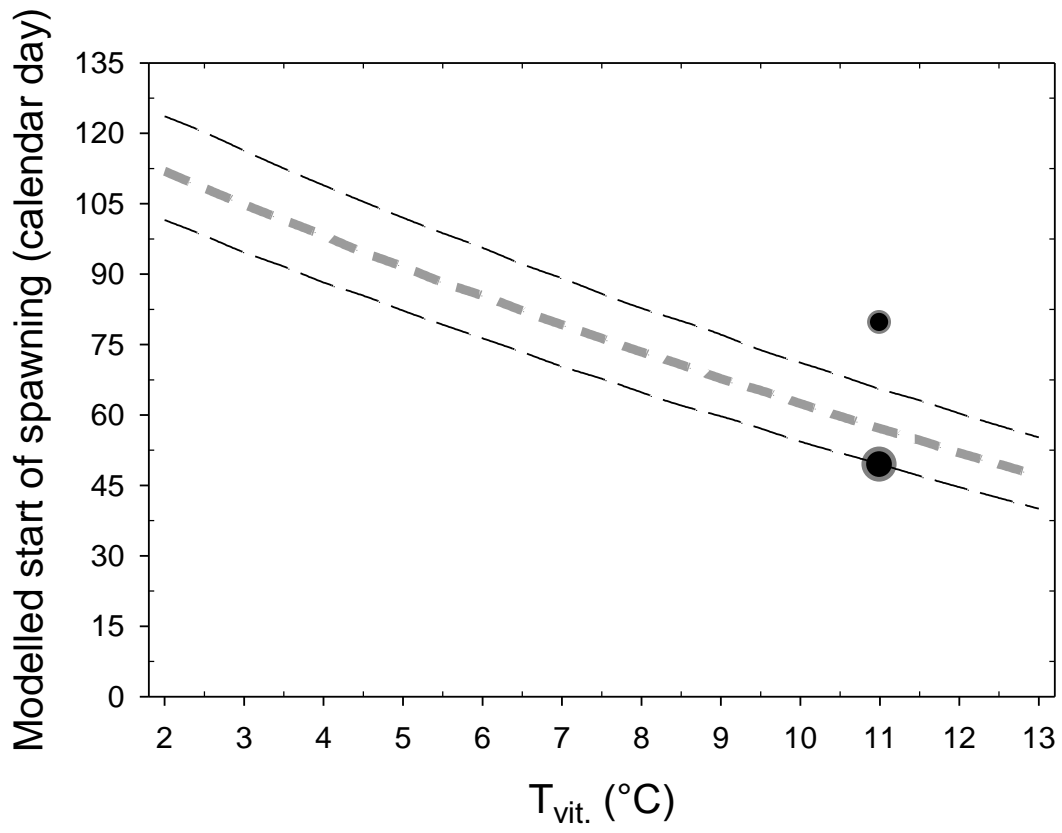


1449

1450 **Fig. 11**

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1453

1454 **Fig. 12**